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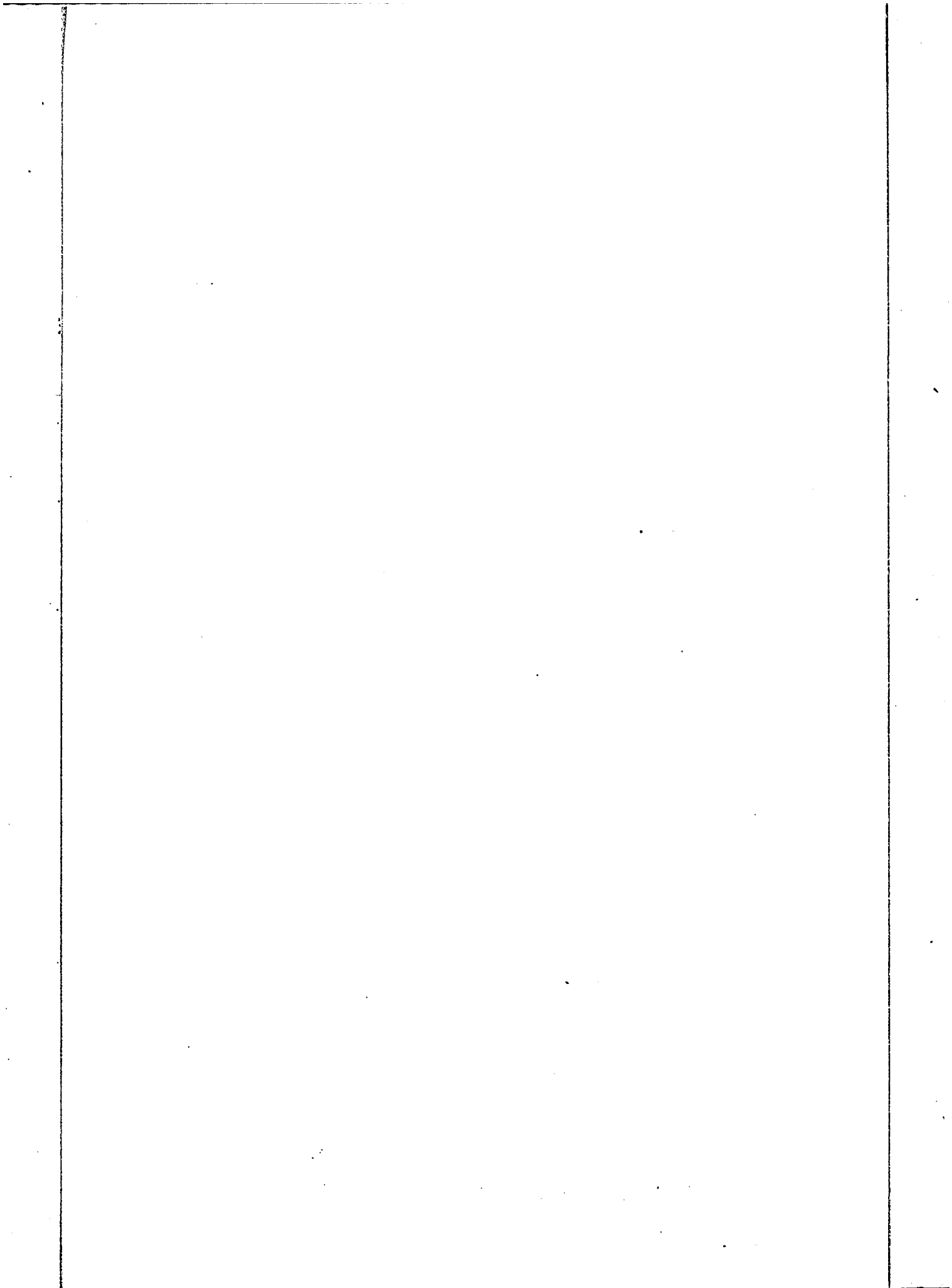
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DYSPLASIA AND CARCINOMA OF THE UTERINE

CERVIX AND VAGINA IN MICE AND RATS

Effects of Minimal Dose Application of 20-Methylcholanthrene



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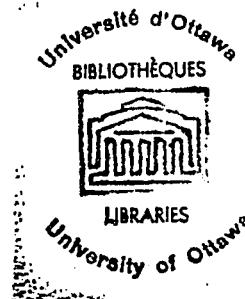
A Thesis

Presented to the University of Ottawa in Partial
Fulfillment of the Requirements for the
Degree of Doctor of Philosophy.

by

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April 1963.



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GENERAL INTRODUCTION

Cancer is usually regarded as an irreversible change in tissue and involves alterations in the direction of growth and differentiation. Whether cancer grows from a single cell or from a number of single cells that have undergone malignant transformation is problematical. This does not, however, alter the basic concept that the malignant cell has been derived from a previously normal cell either directly or by a series of stages and that the cancer cell is the descendant of previously normal cells.

It is difficult to recognize or determine which particular cell will become malignant. Nevertheless, distinctive lesions can often be noted in the affected tissues during the time between the action of the causative agent and the development of the malignant neoplasm. This time interval is referred to as the latent period, and cancer can often be prevented by appropriate treatment of these precursor lesions.

The work described in this thesis was undertaken to study the early cellular changes occurring in the latent period of carcinogenesis and to determine the fate of atypical cells associated with alterations induced by the application of minimal amounts of carcinogen to the uterine cervix of mice and rats.

During the investigation, primary tumours in the lungs and mammary tissues of mice were encountered. These

were considered to result from a remote action of the carcinogen. Other local and systemic effects of the topically applied chemical agents were observed. These included vasculitis in the uterine cervix and in other internal organs and amyloidosis of the spleen, liver, kidneys and adrenals. Hyperplasia of the reticuloendothelial cells with intracellular and extracellular crystalloid materials was observed. These were of sufficient import and interest to include in this report.

The study is, therefore, an investigation of relationships between chemically induced dysplasia and neoplasia of the uterine cervix and vagina in mice and rats with supplementary inquiry into lesions encountered in the course of these experiments.

Induced Dysplasia and Carcinoma
of the Uterine Cervix and Vagina
in Mice and Rats.

INTRODUCTION

During the experimental induction of neoplastic lesions, changes in the mouse cervix antedating invasive carcinoma have been described by several authors.^{15,36,46,53,58,59,62}

Studies in the past several years have indicated the importance of dysplasia (atypical cell hyperplasia) of the cervical epithelium. The significance of this lesion may be summarized as follows:

- a) Dysplastic exfoliates are similar to those of carcinoma in situ;
- b) Dysplasia may occur adjacent to carcinoma in situ or invasive carcinoma;
- c) Dysplasia perhaps develop into carcinoma in situ or invasive carcinoma;
- d) Dysplasias may persist for a long time or it may disappear; and
- e) Dysplasias may be difficult to differentiate histologically from carcinoma in situ.

Dysplastic changes are morphologically atypical and show patterns that suggest aggression but in their immediate effects upon the host they are benign. The occurrence of dysplasia previous to and adjacent to cancer in epithelium treated by carcinogenic chemicals and in a variety of clinical situations suggests that the relationship between these two processes is not fortuitous.^{4,6,11,30,38,43,44,46,64}

An interest in the relationship between dysplasia and carcinogenesis lead to the present investigation.

REVIEW OF THE LITERATURE

Experimental Work

Studies on the association between chemicals and carcinogenesis were ushered in by the work of Sir Percivall Pott⁴¹ in the year of 1775. He observed the relationship between scrotal tumours in chimney sweeps and soot. The importance of a specific extrinsic carcinogen, the existence of a long latent period, and the combination of local factors in determining a susceptible site were realized. A hundred years later, von Volkmann⁶⁰ described the clinical features of industrial dermatitis and cancer caused by coal tar.

In 1889 Hanau¹⁷ painted the scrotum of rats with tar and was able to induce dermatitis but not cancer; had he chosen mice instead of rats he would probably have advanced research in this field by 25 years.

The era of experimental chemical carcinogenesis began with the successful induction of skin cancer with coal tar in the ears of rabbits by Yanagiwa and Ichikawa⁶³ in 1915-1918. Tumours of the skin of mice were similarly induced in 1918 by Tsutsui.⁵⁶

Attempts to induce carcinoma of the uterine cervix and vagina began shortly after the discovery of the carcinogenic properties of crude tar. These were met with sporadic success by methods using direct trauma,³⁵ oestrogen administration^{18,42} and applications of smegma,²³ tobacco tar and other

10,32,45,56,59
known carcinogens. The more consistently positive results were obtained by continuous applications of purified carcinogenic substances to the cervix and vagina.

Studies on carcinogenic effect of various oestrogens have been reported by several workers. Pfeiffer and Allen^{28,53} attempted to induce uterine cervical cancer in monkeys by prolonged exposure to oestrogens and to carcinogenic hydrocarbons. Observations were continued for 9 to 13 years, but cancers were not observed in any of the monkeys. Overholser and Allen³⁵ reported a number of cervical lesions closely resembling early epidermoid carcinoma in monkeys that had received oestrone parenterally. Cervical carcinomas were also observed in mice whose necks and backs had been painted with 1,2,5,6-dibenzanthracene and who were given simultaneous oestrone injection.³⁹ These results indicate that oestrogenic hormones possess some co-carcinogenic effect perhaps by sensitizing the uterine cervix to the carcinogen. However, it has been shown that the prolonged subcutaneous administration of oestrogens for periods of over one year produced cervical carcinomas in various strains of mice,¹⁴ suggesting for oestrogens a more direct carcinogenic action.

¹⁶
Gardner used mice of five different inbred strains to determine effects of systemic or topical application of oestrogens alone or in combination with other steroids. Cancer or premalignant infiltrative lesions appeared in 15

of 17 mice that retained pellets of stilbestrol-cholesterol throughout the course of the experiment. This work constitutes the best evidence to date that oestrogens might act directly as a carcinogenic agent.

Experiments on the possible aetiologic role of human smegma in cervical cancer were reported. While Fishman et al¹⁰ found no tumours in mice after 16 months of intravaginal painting with smegma, Pratt-Thomas et al⁴² and Heins and his colleagues¹⁸ succeeded in producing epidermoid carcinoma by vaginal injections of raw smegma to dba-1 mice.

The early methods of intravaginal application of chemical carcinogens by injection or implantation in the vaginal vault, cervix and lower uterine segment of mice and rats resulted in a few cases of invasive carcinoma.^{7,8,39} This early work was hampered by the use of crude chemical mixtures and inaccurate application of the chemicals to the cervix. Purified carcinogenic substances applied directly to the uterine cervix resulted in a considerably higher yield of epidermoid carcinoma than occurred following either the application of crude tar or the injection of oestrogens. von Haam and Menzies⁵⁸ and von Haam and Scarpelli⁵⁹ were able to produce invasive carcinoma of the cervix in a number of C₃H mice (15 of 80)⁵⁹ by intravaginal painting with 3,4-benzpyrene.

Murphy³² introduced a method of accurate placement

of a carcinogen by inserting a string saturated with methylcholanthrene into the cervical canal under direct visualization. He was successful in producing cervical carcinomas in 27 of 35 strain A mice. By this method, the latent period was shortened to $4\frac{1}{2}$ months.

Dysplastic and neoplastic lesions were also induced in the cervical and vaginal mucosae of C₃H mice by applying crude tobacco tar intravaginally five times weekly for ²³44 weeks. Lesions induced by tobacco tar in the cervix and/or vagina of C₃H mice were similar to those observed during the development of 3,4-benzpyrene-induced cervical carcinoma. Podophyllin in mineral oil applied to the vaginal portion of the uterine cervix of mice and women produced lesions resembling atypical epithelial hyperplasia. ^{19,20,22,29,49} None of these lesions persisted after discontinuation of painting, and no invasion of the stroma was observed. In 1962, ²¹Kaminetzky and McGrew reported epidermoid carcinoma of cervix and vagina in a podophyllin-treated mouse. The mouse had received 120 applications of podophyllin over a 15 month period.

Spontaneous Uterine Cervical and Vaginal Tumours in Mice and Rats.

The relatively high incidence of malignant uterine cervical or fundic neoplasms in man does not appear to have a counterpart in other mammalian species.

Mice:

Very few epithelial tumours of the uterus of untreated mice have been reported and even these are questionable. Gardner¹² stated that only 6 of at least 3,000 untreated mice (0.2%) had upper vaginal or cervical cancer. According to reports by Slye, Holmes and Wells,⁵¹ spontaneous uterine tumours in mice have an incidence of less than 0.06 percent. Uterine cervical cancer was occasionally observed in mice of the PI strain,¹³ but this strain has become extinct because of a high incidence of sterility.

Reports in the literature do not always distinguish clearly between cancer of the cervix and cancer of the body of the uterus in experimental animals. However, spontaneous tumours of the uterine horns in mice are even rarer than those of the cervix.

Rats:

The incidence of spontaneous malignant uterine tumours in rats is very low and only few experimentally induced tumours¹² have been observed.

A review of literature, therefore, indicates that the cervix of mice and rats is susceptible to carcinogenesis in response to the application of carcinogens, oestrogens, among other substances, but that in the mouse and rat, this susceptibility, regardless of strain, is not great and that spontaneous cervical cancer in mice and rats is indeed a rarity.

MATERIALS AND METHODS

Experimental Animals

212 virgin I.C.R. Swiss mice^{XX}, two to three months old, were used in Experiments I and II.

159 virgin phenotypically normal Pituitary Dwarf strain mice^{XXX}, two to three months old, were employed in Experiments III and V.

100 virgin Sprague Dawley rats^{XXX}, two to three months old, were used in Experiment IV.

All animals were fed Master fox cubes and water ad libitum. Temperature in the animal room was maintained between 75-80° Fahrenheit with a relative humidity between 40-60°.

Application of Carcinogen in Experiments I, II, and III (Mice)

Cotton balls of small diameter were moistened with 0.1 ml. of 0.5% 20-methylcholanthrene (20-MC) dissolved in acetone. These were held with an ear dressing forcep and were inserted intravaginally through a 2.0 or 3.0 mm ear speculum until firm resistance indicated contact with the cervix and vaginal vault.

Application of Carcinogen in Experiment IV (Rats) and V (Mice)

A 23 gauge hypodermic needle, 4 cm long, was modified by partially obliterating the pointed end to reduce the patency of the lumen so that it was possible to deliver exactly one drop of the 0.01 ml. of solution under

^{XX} and ^{XXX} Purchased from the Romain Robidoux Laboratory, Saint-Constant, Quebec.

^{XXX} Colony derived from stock purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

gravity or gentle pressure on the plunger. In Experiment IV, carcinogen solutions were applied to rat cervixes under direct vision through a nasal speculum. (Storz No. 1).

In Experiment V, the carcinogen solution was applied to mouse cervixes under direct vision through an otoscopic speculum.

Exfoliative Cytologic Examination

A few drops of normal saline solution were intravaginally pipetted. The admixture of vaginal exfoliate and saline was aspirated and smeared. The smear was fixed immediately in equal parts of ether and 95% ethyl alcohol and stained by the method of Papanicolaou.³⁷

Biopsy

Biopsy specimens were taken from cervixes by means of small biopsy forceps[#] (Figure 1). The tissue was fixed in aqueous formalin 10%, embedded in paraffin, serially sectioned at 3 micra intervals and stained with haematoxylin, phloxine and saffron.

In mice the biopsy procedure was performed blind after visualizing the cervix and vaginal vault with an otoscope. Unfortunately a 2 or 3 mm speculum, the largest the mouse vagina will accommodate without trauma, was too small to accommodate the biopsy forceps. In rats the biopsy procedure was performed through the nasal speculum previously described.

Autopsy

Uteri and vaginae were removed en bloc, pinned out

[#]Hoffman "straight through" forcep, the Storz Instrument Co., St. Louis, Mo., U.S.A.

on small cardboard sheets and fixed in aqueous formalin 10%. The fixed uteri and vaginae of rats were bisected longitudinally in the antero-posterior plane. Each bloc so produced was embedded in paraffin. Not less than 6 step sections cut 3 microns thick were taken at intervals of 6 or 9 microns and were stained with haematoxylin, phloxine and saffron.

Experiments

Five experiments were carried out as outlined below. The strain and number of animals, schedules for treatments and examinations are summarized in Table 1. The carcinogen used was 0.5% 20-methylcholanthrene dissolved in acetone. Controls were treated with acetone or saline alone.

Experiment I

This study was undertaken to observe initial cellular response and patterns of proliferative changes after carcinogen treatment.

56 I.C.R. Swiss mice were treated with carcinogen and 56 with acetone by the method outlined above. Total amounts of 1.0 ml. of 0.5% 20-methylcholanthrene in acetone or acetone alone were applied to each mouse. Applications were made on 10 occasions over a 6 week period. The first 2 applications were separated by a 14-day interval. Pairs of treated and control mice were killed at intervals specified in Table 2 on page 20.

Table 1. Summary of Experiments I,II,III,IV and V.

Experiment	Group	Total No.	Treatment	Schedule of Applications	Total Amounts of Applications	Schedule and Type of Examination
I I.C.R.Swiss Mice	A	56	0.5% MC in acetone	0.1 ml. twice weekly 10 applications	1.0 ml.	Autopsy of animals sacrificed at intervals.
	B	56	Acetone			
II I.C.R.Swiss Mice	A	50 (10 Gr. of 5)	0.5% MC in acetone	0.1 ml. twice weekly from 2 to 11 appl.	from 0.2 to 1.1 ml.	Autopsy following full life span.
	B	50 (10 Gr. of 5)	Acetone			
III Pituitary Dwarf Strain Mice	A	20	0.5% MC in acetone	0.1 ml. twice weekly 10 applications	1.0 ml.	Biopsy. Autopsy following full life span.
	B	10	Acetone			
	C	10	Saline			
IV Sprague Dawley Rats	A	20	0.5% MC in acetone	First Series of appl. 0.02 ml. twice weekly 10 applications	1.8 ml.	Serial biopsies during full life span. Autopsy. (20 rats followed without biopsy for full life span)
		10	Acetone	Second Series of appl. (270 days later) 0.08 ml. every day 20 applications		
		10	Saline			
		10	0.5% MC in acetone			
V Pituitary Dwarf Strain Mice	A	20	0.5% MC in acetone	First Series of appl. 0.02 ml. twice weekly 30 applications	2.2 ml.	
		10	Acetone	Second series of appl. (235 days later) 0.08 ml. every day 20 applications		
		10	Saline			
V Pituitary Dwarf Strain Mice	B	49	0.5% MC in acetone	0.02 ml. twice weekly 10 applications	0.2 ml.	Autopsies of animals.
		24	Acetone			
V Pituitary Dwarf Strain Mice	B	32	0.5% MC in acetone	0.02 ml. twice weekly 30 applications	0.6 ml.	
		14	Acetone			

Experiment II

This study was undertaken to observe dose-response variations that might be encountered in epithelial reactions of the cervix to the carcinogen. Animals were kept for their life spans. 50 I.C.R. Swiss mice for carcinogen treatment and 50 for exposure to acetone were divided into groups of five so as to provide 10 paired groups of test and control animals. Each paired group of animals were given from 2 to 11 applications of 0.1 ml. of 0.5% 20-methylcholanthrene in acetone or acetone alone over a 6 week period.

Experiment III

Forty phenotypically normal Pituitary Dwarf strain mice were divided into three groups. Group A comprised of 20 mice treated with 0.1 ml. of carcinogen twice weekly for five weeks. Group B comprised of 10 mice treated with acetone. Group C comprised of 10 mice treated with normal saline. Cervical biopsies and vaginal exfoliates were examined on the 20th, 40th and 60th days after the initial application. Cervical biopsies were discontinued after the 3rd series of biopsies because of prohibitive complications. The mice were kept for their life spans.

Experiment IV

100 Sprague Dawley rats were divided into two equal groups designated A and B.

These groups were again divided so that in group A, 30 rats were given 10 bi-weekly 0.02 ml. applications of

20-methylcholanthrene. 10 rats were similarly treated by acetone alone, and 10 rats were treated by saline alone.

In group B, 30 rats were given 30 bi-weekly applications of single doses of 20-methylcholanthrene. 10 control animals were given 30 applications of acetone and 10 were treated with 30 applications of saline.

When group A rats and group B rats showed no dysplastic response after 270 and 235 days respectively, the carcinogen treatments were repeated at a level of 0.08 ml of methylcholanthrene daily, for 20 days. Thus, methylcholanthrene-treated rats of group A were given a total of 1.8 ml of the methylcholanthrene solution while those of group B were given a total of 2.2 ml. Controls were given a similar schedule of treatment with acetone or saline.

Exfoliative cytologic examinations and cervical biopsies were performed once every 30 days in 5 rats in each of the experimental and control groups. Thus 15 rats in group A and 15 in group B were so examined. If any rats showed evidence of dysplasia, all rats in all groups were examined at monthly intervals thereafter. 10 rats from each group given methylcholanthrene were followed by exfoliative cytologic studies. These served as controls for biopsy-induced artefact. Necropsies on the rats were made at termination of life spans.

Experiment V

49 phenotypically normal Pituitary Dwarf strain mice designated group A were given 10 bi-weekly 0.02 ml. applications of 0.5% solution of 20-methylcholanthrene in acetone to the cervix uteri. A similar schedule of treatments with acetone in similar volumes was carried out in 24 mice.

32 phenotypically normal Pituitary Dwarf mice designated group B were given 30 bi-weekly 0.02 ml. applications of methylcholanthrene to the cervix uteri, while a similar schedule of acetone applications was carried out in 14 mice of the same strain.

Two methylcholanthrene-treated mice from group A, two methylcholanthrene-treated mice from group B, and one control mouse from each group were killed at monthly intervals.

Histologic Criteria Used for the Diagnosis of Basal Cell
Hyperplasia, ^{Dysplasia,} Pseudoepitheliomatous Hyperplasia, Carcinoma
in Situ and Invasive Carcinoma. 30,33,34,43

A. Basal Cell Hyperplasia.

Basal cell hyperplasia is a term used to describe the multiplication and heaping up of the basal cells of the cervical epithelium. Some maturation does persist and there remain a few layers of mature and cornified epithelial cells on the surface.

B. Dysplasia.

Dysplastic cells are characterized by cytomegaly and pleomorphism with or without cytoplasmic vacuolization, karyomegaly with or without nuclear pleomorphism. Multi-nucleated cells are few. Nuclear hyperchromatism is frequently accompanied by irregular coarse clumping of chromatin. Mitotic figures may be numerous but are infrequently atypical. The dysplastic cells are recognizable as atypical juvenile forms of basal epithelium, although atypical spinous and transitional cells are sometimes present. In dysplastic epithelia, the abnormal cells are haphazardly arranged. In surface epithelium, only partial disorientation of the cell strata occurs, and maturation, while defective and incomplete, is not halted. Basement membranes remain intact.

C. Carcinoma in Situ (Intra-epithelial Carcinoma).

The entire thickness of the involved epithelium is made up of abnormal cells with the complete loss of normal stratification that accompanies maturation arrest. Penetration of the basement membrane is not demonstrable in step or serial sections. The accuracy of the diagnosis depends upon the number of sections examined and the intervals at which they are cut, for basement membrane penetration may be very minute. They are presumed to signal the onset of invasive carcinoma.

D. Pseudoepitheliomatous Hyperplasia.

Irregular downgrowths of epithelium are present but there is the lack of anaplasia and other cellular criteria for the carcinoma. To these criteria the qualification should be added that cancer is not reliably distinguishable from pseudoepitheliomatous hyperplasia except by behaviour.

E. Invasive Carcinoma.

Here the traditional cytomorphologic criteria for malignancy are fulfilled, the basement membrane are breached and infiltrations of the stroma often by clusters and cords of cells are present. The diagnosis of invasive carcinoma is based on the following criteria: a) deep infiltration to the stroma, b) invasion of vascular channels, c) metastasis, and d) cellular anaplasia to a degree inconsistent with pseudoepitheliomatous hyperplasia, with or without abnormal mitotic activity.

OBSERVATIONS

Experiment I

After the first application of the carcinogen, post mortem examinations revealed polymorphonuclear leucocytic infiltrations in the epithelium and stroma with microabscess formations. After subsequent applications, vacuolar and vesicular epithelial changes with necrosis, ulceration, irregular epithelial downgrowths and dysplasia were observed. The distribution of these lesions in cervical and vaginal epithelia in methylcholanthrene-treated mice with reference to time of occurrence is given in Table 2.

Acute inflammations in the cervix uteri and vaginal wall were most marked on the third and fourth day and began to subside at about 3 weeks after the application started. The same degree of inflammation was noted in acetone or saline treated mice.

Degenerative changes with vacuolization and vesiculation of epithelial cytoplasm (Figures 2 and 3) appeared subsequent to the second application of the carcinogen. The most severe degree was observed at about the third week, at which time a focus of epithelial necrosis was found. These changes became inconspicuous after the 5th and 6th weeks. While such changes were frequently observed in methylcholanthrene-treated mice, they were found only occasionally and to a lesser degree in control mice.

Table 2. MC-treated I.C.R. Swiss Mice
(Experiment I) Sacrificed in Pairs.

Day of Sacrifice	No. of Appl.	Vacuolization & Vesicul. of Ep.		Pseudoepith. Hyperplasia		Basal Cell Hyperplasia		Dysplasia		Abnormal Exfoliates (Pap .Class.) ^x
		Vag.	Cervix	Vag.	Cervix	Vag.	Cervix	Vag.	Cervix	
1	1									
3	1									
5	1									
8	1									
11	1									
14	1									
17	2	+		+						
21	3	+		+						
24	4							+		
28	5	##		+						
31	6			+	+			+		
35	7			+						
38	8	+		+	+	+		+		
42	9									
45	10	+		+	+	+		+		II
49	10	+		+	+			##		
69	10	+		+		+	+	+	+	II
100	10				+	+				III
130	10	+	+	+	+	+				II
160	10									II
190	10			+	+	+		+	+	
220	10	+	+	+	+	+	+	+	+	III
250	10	+	+		+	+	+	+		III
280	10							Multiple	+	III
310	10							Multiple		III

1. One symbol (+) indicates involvement of one member of a pair.
Two symbols (++) indicates involvement of both members of a pair.
Symbols (##) and (###) indicate, respectively, 2 and 3 lesions in one animal.
2. x Papanicolaou, G.N. Atlas of Exfoliative Cytology, Harvard Univ. Press, Cambridge, Mass., 1954.

Vasculitis with fibrinoid necrosis of the wall in the cervix was first observed in one of the animals given two applications of methylcholanthrene. Four out of 50 methylcholanthrene-treated mice had this necrotizing vasculitis. One control animal had a similar angiitis in the cervix and vaginal wall.

Irregular downgrowths of epithelium into the cervical and vaginal stroma first appeared in methylcholanthrene-treated animals after two applications and thereafter were frequently seen (Figures 4 and 5). A few control animals had similar changes. These downgrowths were composed of basal cells with variable but limited atypia. Most of the downgrowths were rounded or club-shaped and appeared to be demarcated by a basement-type membrane. In two instances, disorderly finger-like processes extended into the stroma through an inflamed basement-type membrane that was incomplete and indistinct. These lesions were interpreted as pseudoepitheliomatous hyperplasia rather than carcinoma.

Dysplastic lesions were apparent shortly after the 4th applications of the carcinogen and as late as 310 days after applications were begun. Dysplasia of vaginal and/or cervical epithelium appeared in 18 of 34 animals given 4 or more methylcholanthrene applications. Dysplasia did not appear in the control mice nor in mice given less than 4 applications of carcinogen. The dysplastic changes were

characterized by the disorderly orientation of enlarged vacuolated pleomorphic cells, with karyomegaly and minimal hyperchromatism (Figure 6). Mitotic figures were more frequent than in the pseudoepitheliomatous hyperplasia, and occasional atypical mitoses were observed. Atypical exfoliates were found in mice with dysplastic lesions (Figures 8 and 9).

Carcinoma in situ or invasive carcinoma was not noted in any of the mice in this experimental group during the 310 day course of observation.

Five mice given methylcholanthrene had pulmonary adenomas and one mouse had leukemic infiltrations in the spleen, liver, kidneys and lymph nodes. Another mouse was shown to have amyloidosis in the spleen, kidneys, and liver with mesenteric and retroperitoneal abscesses.

Experiment II

Mice given 4 or more applications of the carcinogen had pseudoepitheliomatous hyperplasia, basal cell hyperplasia, dysplasia or invasive carcinoma with or without metastasis. On the other hand, mice given two or three applications of the carcinogen (total amount of 0.3 ml. or less) had neither dysplasia nor carcinoma in the genital region. Control groups given acetone alone had no dysplastic change or carcinoma.

Dysplastic lesions in the cervix and/or vagina were observed in 6 mice. The number of applications of

carcinogen and the longevity of these mice was as follows:

Mouse	No. of Applications	Duration of Life (following application) days
1	4	148
2	4	238
3	9	283
4	9	285
5	10	403
6	11	109

Invasive carcinoma in the cervix and vagina was observed in 6 mice (Figures 7, 10 and 13). The number of applications of carcinogen and the longevity of these mice was as follows:

Mouse	No. of Applications	Duration of Life (following application) days
1	4	374
2	4	452
3	5	155
4	8	385
5	9	285
6	10	278

The invasive carcinomas were solitary greyish-white masses averaging 2.2 cm in diameter (ranging from 1.0-3.8 cm in the greatest diameter) and involving the cervix uteri and upper vagina with extension to the uterine body. One tumour had metastasized to the subcutaneous tissue of the abdominal wall. In two instances, the urinary bladders

were dilated because the neoplasms caused obstruction to the urinary flow. Neoplastic infiltration into the urethra was found on histologic examination in one case. Microscopic examination showed that all carcinomas were epidermoid in type with various degrees of differentiation.

Serial step sections failed to reveal dysplastic changes in vaginal or cervical epithelium of mice with invasive carcinoma except in one case, with dysplastic change in the midportion of the vagina and invasive carcinoma of the cervix.

Pulmonary tumours were observed in 13 methylcholanthrene-treated mice. There were pulmonary tumours in control animals. Mammary tumours were found in 5 methylcholanthrene-treated mice and one mouse of the control group. Amyloid deposits in the kidneys, adrenals, spleen or liver were found in three methylcholanthrene-treated and one acetone-treated mouse. One mouse of the control group had a necrotizing angitis in the uterus and kidneys and healing angitis in the lungs. A thymoma was found in one methylcholanthrene-treated mouse and in one acetone-treated mouse.

Experiment III

Biopsy specimens were initially taken from 40 mice included in the methylcholanthrene-treated groups. Biopsy specimens were adequate but four mice died from peritonitis

attributable to perforation of the vaginal vault and one from haemorrhage at the operative site. After a second series of biopsies in 35 mice, another death was attributable to peritonitis. Three mice subsequently developed recto-vaginal fistulae. After a third series the biopsy procedures were discontinued. Sprague Dawley rats, more suitably for biopsy procedures, were chosen for further experimentation (Experiment IV).

The first biopsies taken 20 days after the initial application, revealed acute inflammations both in methylcholanthrene and acetone-treated animals; vacuolar changes in epithelial cytoplasm were present in one-third of the methylcholanthrene-treated animals. Inflammations were less marked in the 2nd and 3rd series of biopsies. One of the third biopsy series revealed a minute area of basal cell hyperplasia in one methylcholanthrene-treated mouse.

One methylcholanthrene-treated mouse that lived 135 days had pseudoepitheliomatous hyperplasia of the cervix, and another methylcholanthrene-treated mouse that lived 467 days had pseudoepitheliomatous hyperplasia of the cervix and vagina and basal cell hyperplasia of the vagina. No dysplasia or cancer was observed in the genital organs, but one methylcholanthrene-treated mouse had mammary carcinoma. Necrotizing vasculitis of the heart, peri-renal fat, liver, uterine horn and parametrium were encountered in one of the

test group. Angiitis in the cervix was observed in two acetone-treated mice one of which also had amyloidosis in the spleen, liver, adrenals, kidneys and heart.

Experiment IV

Biopsies of rat cervixes under direct visualization at 30-day intervals did not reveal dysplastic or carcinomatous change. Minimal degrees of vacuolar degeneration of cervical epithelium were seen in various rats in the 2nd, 12th and 13th series of biopsies. No basal cell hyperplasia, pseudoepitheliomatous hyperplasia or dysplasia was found in biopsy specimens and no atypia suggestive of dysplastic or carcinomatous lesion were seen in exfoliative cytologic studies.

Death attributed to pyometria, pelvic inflammatory disease and peritonitis occurred after the 2nd series of applications of the carcinogen. Autopsies of 9 rats showed no changes in the cervix or vagina.

One rat developed a subcutaneous, firm mass on the left lateral chest wall that was first noticed on the 217th day after the first application of methylcholanthrene. It was surgically resected on the 249th day, at which time it measured 5 x 4.5 x 3 cm. Histologic examination revealed the tumour to be a mammary fibroadenoma.

Experiment V

Group B mice, given 30 applications of carcinogen,

developed basal cell hyperplasia and epithelial vacuolization between the 55th and 80th day, but these changes were not observed after the 80th day. Dysplastic lesions were first observed on the 55th day and were observed sporadically in the uterine cervix and vagina of mice up to 261 days. Three mice in this group had dysplastic changes in the vagina, and one of these had invasive carcinoma of the cervix. Dysplasia of the cervical epithelium was seen in only one mouse.

Seven mice that lived more than 176 days had invasive carcinoma. Invasive carcinomas of the cervix with direct extension to the uterine body and vaginal vault were observed in 6 mice. One mouse had an invasive epidermoid carcinoma at the vulva involving the urethral orifice. Four of the 6 mice with cervical carcinoma had metastases to lungs, sacral and lower peri-aortic lymph nodes, peritoneum, abdominal wall and ovary (Figures 11 and 12). Three of 6 animals with carcinoma of the cervix uteri had extremely dilated urinary bladders and death in these could probably be attributed to uremia. Microscopic infiltrations of carcinoma into the urethra was seen in these mice. Severe bilateral hydronephrosis and hydroureter was found in one mouse. In four mice with invasive carcinoma of the cervix, the neoplastic process involved the rectum, bladder, urethra and vagina. Histologically, these tumours were

epidermoid in type. One mouse with cervical carcinoma had fibrosarcoma of the vaginal wall. Fibrosarcoma of the cervix uteri was observed in a mouse that lived 326 days. The tumour had metastasized to the left horn, tube and peri-ovarian tissue, pancreas, abdominal wall and peritoneum. Another mouse had a solitary pulmonary adenoma and mammary adenocarcinoma was observed in a mouse that had no significant change in the genital organs.

None of the group A mice that were given 10 applications of the carcinogen showed dysplastic changes or carcinoma in the cervix or vagina during 360 days of observation. Focal nodular hyperplasia of the fibrous elements in the stroma of the cervix was observed histologically in one mouse killed on the 180th day.

Three animals in control groups treated with 10 or 30 applications of acetone had a mild chronic inflammation in the cervix or vagina. There was angiitis of the cervix in two mice, one of which had glomerulonephritis and fibrinoid necrotizing angiitis in the kidneys and a relatively recent splenic infarct. A leiomyosarcoma of the cervix was seen in a control mouse, given 10 applications of acetone, that lived for 420 days after initiation of the experiment.

Table 3. Summary of Main Findings
(Total 269 Mice Examined in Experiments I, II, III & V)

Findings	Exp. I	II	III	V	Total	Days Observed after the Appl.	Metastasis
Dysplasia of Cervix and/or Vag.	18	6		4	28	24-403	
Invasive Ca. of Cervix and Vag.		6		6	12	155-452	5
Pulmonary Tumours	5	13		1	19	160-493	
Mammary Tumours		5 1(control)	1	1	8	65-493	2
Vasculitis in Cervix and Others	4 1(control)	1(control)	1 2(control)	2 2(control)	13	17-360	
Amyloidosis in Various Organs	1	3 1(control)	1 1(control)		6	160-425	
Thymoma		1 1(control)			2	168-346	
Leukemia	1				1	310	Infiltrations of spleen, liver, kidneys & lymph nodes
Sarcoma of Cervix or Vagina				2 1(control)	3	182-420	1
Adenoca. of Jejunum				1	1	360	
Invasive Ca. of Vulva				1	1	326	
Keratoacanthoma of Vulva				1	1	420	

Summary of Observations

The main findings in 163 methylcholanthrene-treated mice and 106 controls are summarized in Table 3.

Of 112 mice that had been given 0.4 ml. or more of 0.5% 20-methylcholanthrene, dysplastic lesions in the cervix and/or vagina were observed in 28 (25%) and invasive carcinoma involving the cervix and vagina were observed in 12 (10.7%). Dysplasia was observed as early as the 24th day and as late as the 403rd day after initial application.

Invasive carcinoma was seen in mice that lived from 155 to 452 days after the first methylcholanthrene application. Serial tissue sections of mice bearing invasive carcinoma in the genital organs showed concomittant epithelial dysplasia in 2 mice while in 10 others there was no accompanying dysplasia. Five of the 12 invasive carcinomas of the cervix metastasized to remote sites.

Sarcoma of the cervix or vagina developed in two methylcholanthrene-treated mice and in one control mouse. A Pituitary Dwarf strain mouse with fibrosarcoma of the uterine cervix had metastastic lesions in the left horn, tube, peri-ovarian tissue, pancreas, abdominal wall and peritoneum.

One methylcholanthrene-treated mouse had invasive epidermoid carcinoma of the vulva and another had keratoacanthoma of the vulva. The pulmonary and mammary

tumours encountered in mice are reported on pages 62 to 88 and 89 to 117 respectively.

Vasculitis resembling polyarteritis nodosa developed in 7 methylcholanthrene-treated and in 6 acetone-treated mice that were given 2 or more applications. In all but one of these mice the uterus was involved; in 10 of 12, the angiitis (vasculitis) was prominent in the cervix uteri. In 5 mice, the vascular lesions were more generalized and involved the heart, lungs, spleen, kidneys, ovaries or peri-renal fat. No vasculitis appeared in saline-treated animals.

The angiitis was necrotizing and involved the smallest branches of arterial and venous vessels. Histologically, active and acute or relatively inactive and chronic inflammation was observed. In an active phase, the blood vessel wall was involved in a fibrinoid necrosis and an intense cellular reaction predominantly of round cells, neutrophils and few eosinophils (Figures 28, 29, 30 and 33). Mural thickening produced obliteration of lumens where fibrinoid necrosis involved entire vessel walls. These changes were accompanied by perivascular cellular infiltrations. An inactive phase was characterized by cellular fibrosis and mural thickening with marked round cell infiltration without necrosis (Figures 31 and 32).

Amyloidosis was found in 4 methylcholanthrene-treated and in 2 acetone-treated mice after 4 or more applications. The amyloid had affinity for congo red dye, stained metachromatically with methyl violet dye and was periodic acid-Schiff positive. It was deposited interstitially in heart, liver, spleen, adrenal glands and kidneys (Figures 34,35 and 36). In the area of amyloid deposition, especially in spleen and liver, there were remarkable increases in numbers of plasma cells and lymphocytes (Figure 37). No amyloidosis appeared in saline-treated animals.

Hyperplasia of peculiarly striated acidophilic reticuloendothelial cells (histiocytes) and crystalloid materials were observed in the lungs, gall bladder and pulmonary hilar lymph nodes of both acetone-treated and methylcholanthrene-treated mice. These are reported in detail on pages 118 to 145.

Sprague Dawley rats given methylcholanthrene (1.8 ml. or 2.2 ml.) had no significant local changes during 390 days of observation.

DISCUSSION

Minimum Dose of Carcinogen for Induction of Dysplasia and Carcinoma.

Mice:

A total of 0.4 ml. solution of 0.5% methylcholanthrene in acetone appears to be the minimum dose for induction of dysplasia and carcinoma in both Pituitary Dwarf strain and I.C.R. Swiss mice regardless of the duration of applications. In I.C.R. Swiss mice of experiments I and II, 0.4 ml. of 0.5% methylcholanthrene in acetone in 4 applications of 0.1 ml. each was a threshold for dysplastic and carcinogenic response in epithelial fields of the lower genital tract. The quantity of methylcholanthrene to produce an effect on the treated epithelium cannot be estimated accurately. In Experiments I and II the carcinogen was applied in minute cotton balls moistened with methylcholanthrene solution and some of the methylcholanthrene must not have reached the epithelial cells. In Experiment V a similar qualification applies, for some of the methylcholanthrene solution, when dropped upon the cervix uteri, must have dispersed and flowed away. Nevertheless, in Experiment V, 0.6 ml. of 0.5% methylcholanthrene solution in 30 applications of 0.02 ml. excited lower genital tract dysplasia or carcinoma in Pituitary Dwarf strain mice, while 0.2 ml. in 10 applications

of 0.02 ml. failed to do so. The threshold of methylcholanthrene in acetone for lower genital tract dysplasias and carcinomas are apparently of about the same order.

Most previous experimental inductions of cervical carcinoma depended upon many repeated applications of a carcinogen for a long period. The present experiments, however, clearly indicate that the minimum dose required to induce carcinoma and dysplasia of the cervix uteri is very minute .

Rats:

To explain the negative response in methylcholanthrene-treated rats, two possibilities are apparent. First, the total dose applied to the rats may have been below the threshold required to induce such changes in this species or time required for its carcinogenic effect to become manifest seem to have been insufficient. Secondly, the Sprague Dawley rat used in these experiments may be resistant to cervical cancer induction by chemical agents.

The literature is not informative as to the susceptibility of experimental induction of uterine cancer in Sprague Dawley rats. Although many experiments concerning chemically induced cervical cancer have been carried out using different strains of mice, similar studies in rats have not been widely undertaken. By painting 9,10-dimethyl-1,2-benzanthracene (DMBA) to the vagina and cervix uteri of

female Black-hooded rats, Cherry and Glucksmann⁵ induced sarcomas of the vaginal wall, minimal hyperplasia and "pre-carcinomatous" lesions of the vaginal epithelium but no carcinoma of the cervix or vagina.

Vellios and Griffin⁵⁷ were initially unable to produce carcinomas in the cervixes of rats (Wistar strain) by suspending a string impregnated with 17,12-dimethyl benz(a) anthracene in the cervical canal. When the procedure was repeated, however, with the use of a knot saturated with beeswax and carcinogen so that the carcinogen might be released more slowly, one third of the rats developed tumours (epidermoid carcinoma, adenoacanthoma and carcinosarcoma) of the cervix and endocervix.

Dysplasia

Dysplasia of the cervix uteri and vagina is considered to be a significant change for the following reasons: (a) it did not appear in control mice, (b) it preceded the occurrence of invasive carcinoma, (c) the dose of carcinogen required to induce the dysplastic lesion also produced carcinoma in the cervix and vagina of mice, and (d) dysplasia observed in the experimental mice was similar to that seen in the human cervix which at times accompanies both in situ and invasive carcinoma.⁴⁴

Many investigators^{6,32,46, 59} who have produced experimental carcinoma of the cervix in mice have described epithelial

changes which nearly always preceded invasive carcinoma. The present experimental works confirm these findings.

The appearance of dysplastic cells was usually preceded by an inflammatory reaction which began a few days after the administration of the carcinogen. The inflammatory process usually had reached its peak and had begun to subside at the time cellular dysplasia became apparent. Cellular abnormalities were found in parabasal and intermediate layers and were less distinct in the superficial cells. Cytoplasmic changes were more noticeable than nuclear changes and consisted of the appearance of cytoplasmic granules or vacuoles, and enlargement of the cells. Nuclear changes of experimental cervical dysplasia consisted primarily in swelling of the nucleus. The nucleus often assumed a vesicular appearance, but there was no marked nuclear hyperchromasia or abnormal nucleoli.

Definite information as to the reversibility or irreversibility of dysplasia cannot be given. There is no doubt, however, that some of the dysplastic cells persisted for a long time. Dysplastic lesions appeared on the 24th day after initial application and were evident as long as 368 days after the discontinuation of carcinogen application. Nevertheless the minimal degrees of dysplasia may be reversible and it is possible that as long as the changes are confined primarily to the cytoplasm the lesions are frequently reversible. The possible reversibility of chem-

ically induced dysplastic changes in the cervix and vagina is comparable to the regression of some of the skin lesions such as keratoacanthoma and squamous papilloma after the discontinuation of carcinogen applications.

In follow-up biopsies of 242 selected cases of atypical hyperplasia in humans, Peckham and Greene³⁸ found that 40.5% disappeared, 10.7% were associated with adjacent carcinoma in situ, and 48.8% persisted. On the basis of cytologic studies of cervical scrapings from 209 human cases with dysplastic lesions, Sağiroğlu⁴⁸ concluded that about 10% of the cases were progressive to cancer, 11% were regressive to normal and the remaining 79% were producing precancer cells at the end of a 5 year follow-up.

There is a close relationship between the occurrence of dysplastic cells and invasive carcinoma. In the present experiments, dysplastic lesions preceded invasive carcinoma by 130 days and dysplastic lesions were the only change which persisted throughout the observation until invasive carcinoma appeared. The concomitant dysplastic lesions were found in 2 of 12 mice bearing invasive carcinoma. The relationship of dysplasia and invasive carcinoma in the human cervix was studied by Reagen et al⁴⁴ who noted that dysplasia on the average occurred some 6 years earlier than did in situ carcinoma in humans. Patients with atypical epithelial hyperplasia of the cervix had a high incidence of in situ and invasive cervical carcinoma, especially if the

abnormal cells occupied two-thirds or more of the thickness of the epithelium.²²

Perinuclear Halos of Dysplastic Cells

In these experiments, cytoplasmic vacuolization of degenerating and dysplastic epithelial cells are morphologically similar to the vacuolization occurring in arsenical keratosis and carconima of the skin, some cells in Bowen's disease,²⁶ and Paget's cells in both extramammary or mammary lesions.²⁷

Vacuolization and ballooning in the upper layers of the epithelium are commonly encountered during epithelial dysplasia. These changes are referred to as "koilocytotic atypia",²⁴ "wartyp atypia"⁵² and "perinuclear halo".¹ Although cytoplasmic vacuolization and ballooning in cells have been illustrated and described as part of a "precancer cell complex", the significance of the perineuclear vacuoles is not clear. Occasionally, glycogen can be demonstrated by special stains in single cells, but generally these cells are not rich in glycogen.²⁴ While Sađirođlu⁴⁷ expressed the opinion that the halo is a mechanically produced artefact, this view is not widely held since the halo can be demonstrated by a variety of techniques, including electron microscopy.²⁴

The halo cavity may well be filled with fluid. One of the basic physiochemical differences between normal and cancer cells is the greater water content of tumour tissues.⁵⁴

Broghamer and Christopherson³ made an interference microscopic study of aspirations from the vagina of mice whose cervixes were treated with 20-methylcholanthrene. Whereas normal fresh cells were twice as large as were alcohol-ether-fixed cells from normal animals, fresh cancer cells had an average area 8 times as great as that of fixed cancer cells. These findings indicate a marked increase in alcohol-ether-extractable substances in cancer cells, with a relatively larger proportion of this material in the cytoplasm.

Ayre¹ quoted Cowdry and Gey as suggesting that the halo is intranuclear and is produced by virus action. Thierry et al⁵⁵ in 1959 described virus-like particles in chemically induced carcinoma of the uterine cervix of C₃H/A mice treated with bi-weekly applications of 3,4-benzopyrene. They suggested that virus-like particles were a result, rather than the cause, of the carcinomatous process. Virus particles⁵⁵ are an unusual finding in chemically induced tumours, although they are found in mammary carcinoma known to carry the milk factor.²

Vacuolization of the cytoplasm or, occasionally, of the nucleus is also described as the immediate effect of radiation on the squamous epithelia of the cervix and the vagina.²⁵

Thus, the perinuclear halo and ballooning changes

that occur in dysplastic cells in the course of carcinogenesis appear to be the reflection of cellular damage and metabolic changes.

Carcinoma in Situ

Carcinoma in situ was not induced in these experiments. This lesion has been previously reported by some authors⁵⁹ whereas others⁶ indicated that invasive cancer was preceded by dysplasia but a stage equivalent to in situ carcinoma of humans was not noted. This important distinction may, however, merely reflect difference in interpretation of histologic sections.

The concept that carcinoma in situ in humans is a stage in the development of clinically invasive carcinoma is well recognized. Whether or not progression from in situ to invasive carcinoma is inevitable if the process is left undisturbed has not yet been determined. Nevertheless, there are many reasons to assume this sequence. The incidence of carcinoma in situ and that of true carcinoma is about the same.⁵⁰ If carcinoma in situ is the pre-invasive stage of cancer, the age of the patient should be younger than of the patient with invasive cancer. It has been reported that 38 years is the mean age for carcinoma in situ,⁶⁴ 48 years for invasive carcinoma.⁹ Women in four groups studied by Fidler and Boyes were as follows:

1. Carcinoma in situ - 41.7 years (473 cases)

2. Carcinoma with discrete micro-invasive foci -
46.0 years (31 cases)
3. Occult invasive carcinoma with confluent in-
vasive foci - 51.0 years (20 cases)
4. Clinical invasive carcinoma - 52.4 years
(512 cases).

Carcinoma in situ of the cervix is six times⁵⁰
and invasive carcinoma of the cervix is five times⁶¹ more
common in non-Jewish women than in Jewish women. In tissue
culture studies Zinser⁶⁵ found that there were no apprec-
iable differences between transplanted tissue from a car-
cinoma and that from a carcinoma in situ and confirmed the
similarity of growth properties of invasive carcinoma and
of carcinoma in situ.

SUMMARY AND CONCLUSIONS

Although spontaneous cervical cancer in mice and rats is a rarity, a review of the literature indicates that the cervix of mice and rats is susceptible to dysplastic and carcinomatous changes induced by the application of known chemical carcinogens, oestrogens and smegma.

When the carcinogen is applied to the uterine cervix in mice, the lesions seem to progress through the stages of acute inflammation, degeneration of epithelial cells characterized by vacuolization and vesiculation of cytoplasm, basal cell hyperplasia, pseudoepitheliomatous hyperplasia, varying grades of dysplasia and finally to invasive carcinoma with occasional distant metastases.

Dysplasia was followed by invasive carcinoma but carcinoma in situ comparable to that seen in the human cervix was not noted. Dysplastic lesion appeared in the cervix or vagina as early as 24 days after the initial application. Invasive carcinoma of the cervix and vagina started to appear 155 days after the first application.

Of 112 mice that had been given 0.4 ml. or more of 0.5% 20-methylcholanthrene dissolved in acetone, dysplasia in the cervix and/or vagina were observed in 28 mice (25%) and invasive carcinoma involving the cervix and vagina were observed in 12 mice (10.7%). No carcinoma or dysplastic lesion developed in acetone-treated or saline-treated control animals.

The minimum dose that induced both dysplasia and carcinoma in the uterine cervix and vagina of I.C.R. Swiss or Pituitary Dwarf strains of mice was 0.4 ml. of 0.5% 20-methylcholanthrene.

Sprague Dawley Rats given methylcholanthrene (1.8 ml. or 2.2 ml.) had no significant local changes during 390 days of observation by series of biopsy, exfoliative cytological examination and autopsy. The amounts of methylcholanthrene or time required for its carcinogenic effect to become manifest seem to have been insufficient.

Data as to the reversibility or irreversibility of dysplastic lesions is not given but it was established that some dysplastic cells may persist as long as 368 days after the discontinuation of carcinogen application.

Figures 1, 2, and 3

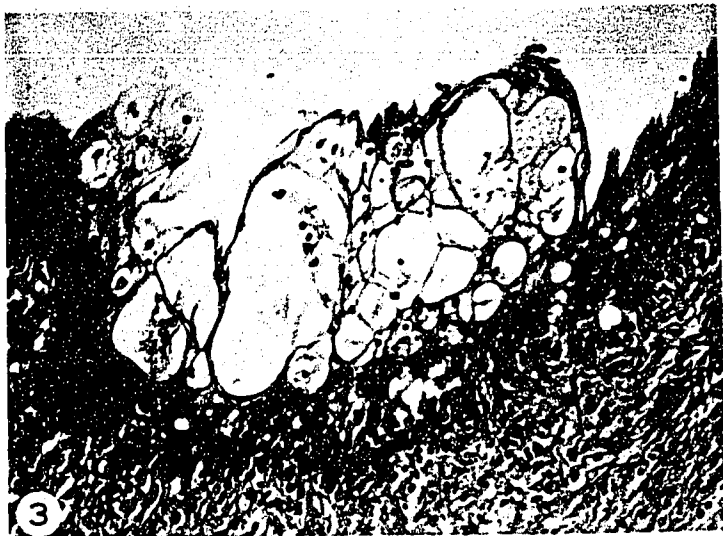
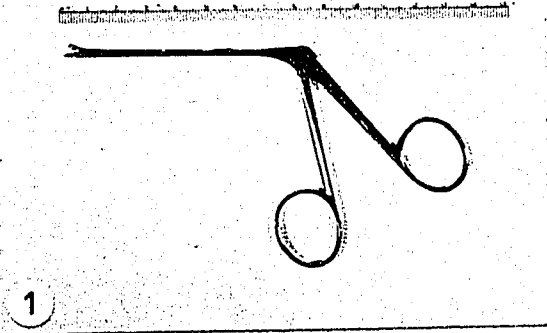
All sections are stained with haematoxylin, phloxine and saffron unless otherwise stated.

Figure 1. The biopsy instrument.

(Hoffman "straight through" cutting forcep).

Figure 2. Vacuolization of epithelial cells appeared 17 days after methylcholanthrene was first applied. Experiment I. X250.

Figure 3. Vesiculation of epithelium appeared 21 days after methylcholanthrene was first applied. Experiment I. X125.



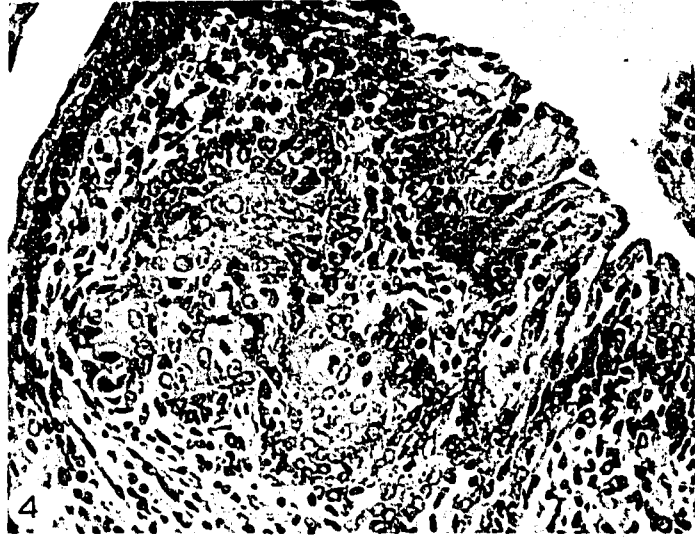
Figures 4, 5 and 6

Figure 4. Pseudoepitheliomatous hyperplasia of the cervix uteri. Experiment I. X145.

Figure 5. Pseudoepitheliomatous hyperplasia of the vagina. Experiment I. X65.

Figure 6. Dysplasia of cervical epithelium.

Figure 6. Dysplasia of cervical epithelium. Experiment I. Large cells with vacuolated cytoplasm are arranged in a disorderly fashion. Toward the surface the flattening out of cells indicates that the capacity to stratify and mature is not wholly lost. The abnormal epithelium blends into the bordering healthy mucosa to the right of the field without a sharp line of demarcation. X125.



Figures 7,8,9, and 10.

Figure 7. Dysplasia of cervical epithelium.

Experiment II. Atypical cells are characterized by abundant cytoplasm showing ballooning degeneration and large nuclei. Note that not all layers are involved despite an abundant population of atypical cells. X110.

Figure 8. Atypical vaginal exfoliates.

Experiment I. Atypical cells are irregular, occasionally tadpole-shaped. Papanicolaou stain. X260.

Figure 9. Atypical vaginal exfoliates.

Experiment I. Nuclear pleomorphism and karyomegaly is evident. Papanicolaou stain. X425.

Figure 10. Epidermoid carcinoma of the cervix and vagina.

Experiment II. This I.C.R. Swiss mouse was given 9 methylcholanthrene applications and lived 285 days. The carcinoma is principally exophytic. Dilatation of the urinary bladder apparently reflects obstruction of the urethra from extrinsic pressure and invasion by the neoplasm.



Figures 11A and 11B

Figure 11A. Invasive carcinoma of the cervix uteri.
Experiment V. The Pituitary Dwarf strain mouse was given 10 methylcholanthrene applications and lived 261 days. The uterus is bisected. The mass in the upper right is an ovarian metastasis. The corpus uteri is filled with necrotic debris.

Figure 11B. Sectional aspect of bisected uterus of Figure 11A.
Experiment V group B. Uterus and vagina, urinary bladder, urethra and rectum were removed en bloc. Carcinomatous extension into parametrium and into the uterine corpus is evident. X5.



Figures 11C, 11D and 11E.

Figure 11C. Invasion of rectum by epidermoid carcinoma of cervix.

Detail of Figure 11B - Experiment V group B. The cancer invades the wall of the rectum to the level of the mucosa. X52.

Figure 11D. Invasion by epidermoid carcinoma into skeletal muscle of abdominal wall.

Experiment V group B. X115.

Figure 11E. Epidermoid carcinoma of the cervix, metastatic to peri-aortic lymph node.

Experiment V group B. Neoplastic cells are well differentiated. X 50.



Figures 12A, 12B and 13.

Figure 12A. Epidermoid carcinoma of the cervix with direct extension to the uterine body and vaginal wall and metastasis to right lung.

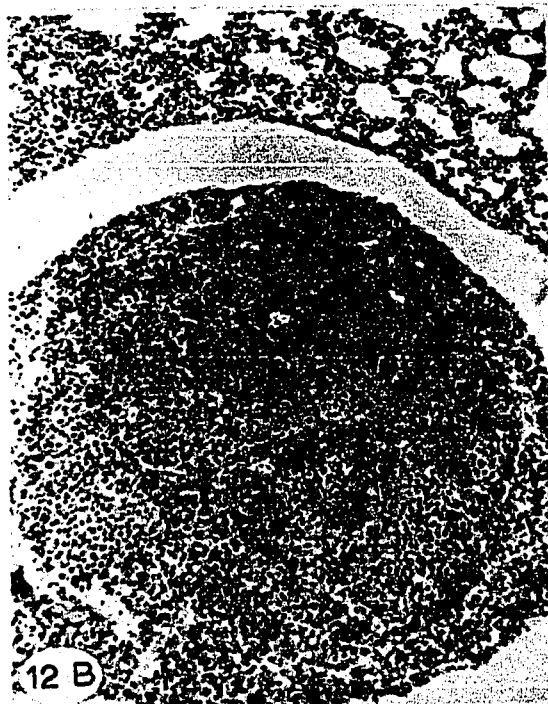
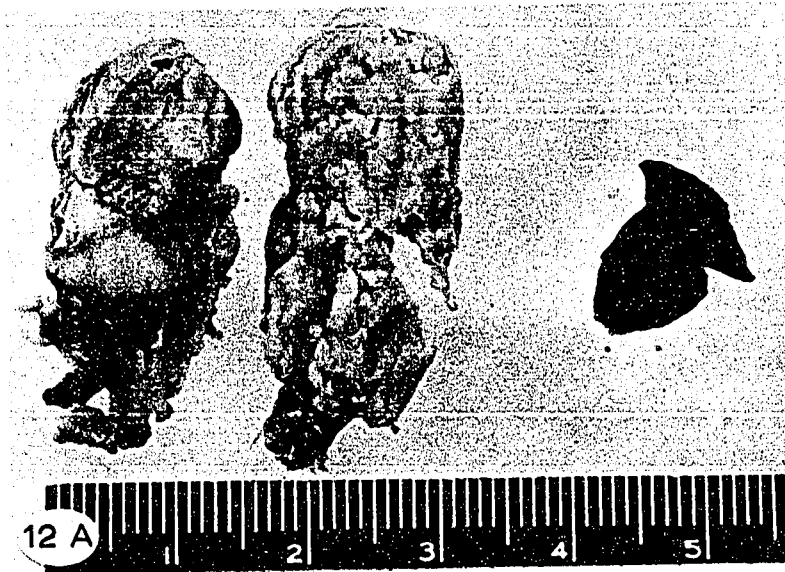
Experiment V group B. The uterus is bisected; to the right is the lung. The Pituitary Dwarf strain mouse lived 269 days after the first of 30 methylcholanthrene applications.

Figure 12B. Epidermoid carcinoma of the cervix uteri, metastatic to lung.

Experiment V group B. Microscopic appearance of the lesion illustrated in Figure 12A. X115.

Figure 13. Carcinomatous mass involving sectioned uterus and upper portion of vagina.

Experiment II. I.C.R. Swiss mouse was given 4 methylcholanthrene applications and lived 374 days.



Primary Pulmonary Tumours in Mice
Following the Application of
20-Methylcholanthrene to the
Uterine Cervix.

INTRODUCTION

The application of chemical carcinogens to mice of a susceptible strain either by methods of inhalation, intratracheal injection or by application to a site remote from the lung increases the incidence of primary pulmonary neoplasms. ^{72,98,104,113} Murphy and ¹⁰⁴Sturn discovered that the application of tar to the skin increased the incidence of lung tumours without leading to skin tumours when in each mouse, a different area of skin was treated in each of the 12 carcinogen applications. Since then, many investigators have observed the action of carcinogens upon tissues remote from sites of application.

In this study, primary pulmonary neoplasms were observed in two different strains of mice treated with minimal intravaginal doses of 20-methylcholanthrene. Various aspects of this neoplasm are presented on the basis of examination. A total of 102 such tumours in 19 animals was observed.

REVIEW OF THE LITERATURE

Spontaneous Lung Tumours

The lung is the commonest site of spontaneous tumour formation in the mouse. The incidence varies from strain to strain, from 4% to over ^{81,116}90%. These tumours are usually alveolar adenomas and adenocarcinomas. Spontaneous bronchogenic squamous cell carcinomas resembling those in man have rarely been found in rodents.

The first description of a spontaneous lung tumour in the mouse was by Livingood ⁹⁰ in 1896. Tyzzer ¹²¹ in 1908 drew attention to the frequency of lung tumours in mice. Slye, Holmes and Wells ¹¹⁶ found lung tumours in 163 of 6,000 mouse autopsies (4%); of the 163 mice with lung neoplasms, 63 (38.6%) ¹¹⁶ were believed to be malignant. Slye et al pointed out that lung tumours rarely appeared before the age of twelve months. Lynch ⁹³ found no tumours in mice under the age of 8 months, and noted the greatest incidence at 24 months.

Sex had no appreciable influence. Bittner ⁶⁸ found that the incidence of pulmonary tumour is approximately the same in males as in virgin females of inbred A strain of mice.

Lynch ⁹⁴⁻⁹⁶ crossed strains of mice with a high and low incidence of spontaneous lung tumours and obtained convincing results that susceptibility to the development of these tumours is inherited. She concluded that there are constitutional types of mice differing in susceptibility to

lung tumours and that these differences are organ-specific and inherited. Heston and Dunn⁸¹ observed that the incidence of spontaneous pulmonary tumours in strain A mice was 50 to 90% while strain L mice appeared to be resistant to the development of pulmonary tumours.

The spontaneous pulmonary tumours reported thus far were adenomas and adenocarcinomas except for the single instance of a peripheral epidermoid carcinoma.¹²² These¹²⁰ tumours were considered to originate from either alveolar¹¹⁶ or bronchial epithelium. The tumours are often situated close to the pleura and may be single or multiple.

Lung Tumours Induced by Chemical Compounds

Lung tumour produced by carcinogenic agents may be divided under two groups -- those produced by the direct introduction of carcinogenic agents into the lung and those produced by application or introduction of carcinogenic agents at a remote part of the body.

Compounds used for inhalation methods are road dust⁷⁰ "freed" from tar products,¹¹¹ coal smoke soot and ozonized gasoline.⁸⁸ Andervont⁶⁶ applied carcinogen directly by transfixing lung tissue with silk thread coated by 1:2:5:6-dibenzanthracene.¹¹³ Shimkin produced pulmonary adenomas by intratracheal injection of dibenzanthracene.

Other methods employed for chemical induction of pulmonary tumours are painting the skin,¹⁰⁴ subcutaneous injections,^{66,101,102} intravenous injections,¹¹³ intrasplenic

injections,⁷⁶ introduction of carcinogens into the rectum,¹¹⁰
vagina, peritoneal cavity¹¹⁰ and into stomach by intubation⁹⁸
or oral feeding.¹⁰⁰ By these methods, and with agents such
as 4-nitroquinoline N-oxide, 20-methylcholanthrene, tar,
isonicotinic acid, hydrazid, dibenzanthracene, benzpyrene
and urethane, the incidence of pulmonary tumours ranged
from 8.1 to 100%, that of control animals from 3 to 20%.

Shimkin and McClelland¹¹⁵ showed a dose-effect
relationship. They injected 20-methylcholanthrene intra-
venously into the tail veins of strain A male mice and
found that the increase in numbers of pulmonary tumours
was related to an increase in the dose and to time elapsed
after carcinogen injection. The induced tumours were in-
variably multiple and both lungs were sometimes extensively
but randomly riddled with neoplasms.

MATERIALS AND METHODS

A total of 269 mice in Experiments I, II, III and V were studied. 106 served as controls. Methods of carcinogen application are described on pages 10 to 16.

Autopsy

The numbers and size of pulmonary nodules observed grossly were recorded and lungs were embedded in toto for serial sections. All sections were cut at 3 micron intervals, and stained routinely with haematoxylin, phloxine and saffron dyes. Selective pulmonary lesions were stained with Mayer's mucicarmine, alcian blue, periodic acid-Schiff, Gomori's aldehyde fuchsin, sudan III, oil red O, and Wilder's reticulum stains.

OBSERVATIONS

Eighteen of 83 (22%) I.C.R. Swiss mice which were given applications of methylcholanthrene had single or multiple neoplasms in one or both lungs. None of the control I.C.R. Swiss mice had such lesions. One of 80 (1.3%) methylcholanthrene-treated Pituitary Dwarf strain female mice had a pulmonary tumour at 270 days of age. Control Pituitary Dwarf strain mice had no pulmonary neoplasms. In 19 methylcholanthrene-treated mice with pulmonary tumours, carcinoma of the cervix and/or vagina occurred in only 3 mice, and epithelial dysplasia of the cervix and/or vagina occurred in only 2 mice. Fourteen mice showed no lesions at the site of methylcholanthrene application.

One mouse treated with 20-methylcholanthrene had three primary neoplasms: mammary carcinoma, invasive carcinoma of the upper vagina and multiple pulmonary adenomata with malignant change in one of adenomatous lesions.

The pulmonary neoplasms were spheroid and varied from microscopic size to 6 mm. in diameter. The grossly visible tumours were greyish-white, soft, mainly peripheral (Figure 14). The pleura close to the nodules was slightly thickened and when the underlying tumour was large, the pleura was umbilicated. In 13 of 19 mice these lesions were multiple varying in number from 2 to 35; in 11 mice they were bilateral.

Microscopically, the lesions were non-encapsulated, composed of cuboidal or columnar epithelium arranged in acinic and papillary patterns (Figure 15). The bordering lung parenchyma was compressed indicating degrees of expansive enlargement. Tumour cells appeared to be invading on broad fronts and engulfing adjacent pulmonary alveoli and bronchiolar lumens. The cytoplasm of the cells was slightly acidophilic and devoid of cilia. The nuclei were single, round or oval with few mitotic figures. The tumour cells were usually supported by a sparse delicate fibrous stroma. Two lesions revealed exaggerated fibrous changes of the stroma and partial replacement of tumour cells by fibrous tissue (Figure 17). One lesion contained a necrotic focus and collections of inflammatory cells (Figure 18). Multiple foci of hyperplastic alveolar lining cells were frequently found in the lung with fully developed tumour nodules (Figure 19).

An attempt was made to separate the pulmonary neoplasms by histologic criteria into benign and malignant categories, but in no instance were extrapulmonary metastases from any of these lesions observed. Of 102 tumours, 4 lesions, in two I.C.R. Swiss mice, showed evidence of malignant changes characterized by anaplasia, hyperchromatism, frequent mitosis and disorientation of cells (Figure 20). Neoplastic cells were seen in the lumen of a blood

vessel at the periphery of the tumour (Figure 21) and many tumour emboli were seen in the blood vessels of both lungs (Figure 22).

The fibrous stroma supporting groups of tumour cells revealed small amounts of reticulum and no elastic fibres. Lack of mucin in the tumour cells was demonstrated by the constantly negative results with the periodic acid-Schiff reaction, alcian blue and mucicarmine stains. Occasionally, however, small amounts of PAS positive substances were found in the lumens of alveloid spaces in some tumour nodules. Sudan III and oil red O stains for fats indicated that occasional cells contained a few lipid granules.

DISCUSSIONS

The incidence of primary pulmonary tumours in mice treated with 20-methylcholanthrene to the cervical os is interesting because the site of tumour origin does not correspond to the site where the concentration of introduced carcinogen is apparently greatest, and because pulmonary tumours were often induced in the absence of cancer at the site of application. Carcinogen or carcinogenic metabolites may have been reached the lungs through lymphatics or veins, and lungs may have been more susceptible than the lower genital tract to minimal carcinogenic stimuli.

In these studies, primary pulmonary tumours were induced in 18 of 83 (22%) I.C.R. Swiss mice and in 1 of 80 (1.3%) phenotypically normal Pituitary Dwarf mice. Lynch^{96,97} stated that her attempts to induce cancers in the lungs of mice indicated that strains differ in susceptibility to induced tumours, and that strains less susceptible to spontaneous growths also were less susceptible to tar-induced tumours. Carcinogenic tar in lampblack failed to induce carcinoma of the lung in any of 100 genetically tumour-resistant mice (C₅₇ Black strain)¹¹² while an 8% incidence of lung tumours in a less resistant strain of mice with chimney soot¹¹¹ was obtained by Seeling and Bonignus.⁸¹ Heston and Dunn stated that intravenous injection of 0.5 mg. of 1,2,5,6-dibenzanthracene produced 100% incidence of pulmonary tumours

in strain A mice, which are highly susceptible to lung tumours, and a 24% incidence in strain L mice, which are extremely resistant to the spontaneous development of such tumours.

81

Heston and Dunn showed the precise site of action of the genes controlling the production of pulmonary tumours. They concluded that most of the genic action is localized in the lung tissue itself. Lung tissues from strain A and from strain L were transplanted subcutaneously into the F₁ hybrid of the two strains. Following the intravenous injection of dibenzanthracene into the hosts, pulmonary tumours were found in 39.1% of the transplants from strain A donors and in 3.6% of the transplants from strain L donors. Thus, a large part of the difference in genetic susceptibility to pulmonary tumours of the donor strains was retained in the transplanted tissue, suggesting that the action of at least most of the susceptible genes by which these two strains differ is localized in the lung tissue.

There is no morphologic difference between spontaneous and chemically induced tumours, between tumours induced in different strains of mice, between tumours produced by different carcinogenic chemical compounds, between tumours induced by different routes of injection, or between tumours induced by the same carcinogens in different media.

The histogenesis of chemically induced pulmonary tumours in mice has been a point of controversy. Magnus⁹⁸ and Orr^{107,108} suggested the origin of these tumours from bronchial or bronchiolar epithelium, whereas Furth and Furth,⁷⁶ Campbell,⁷⁰ Grady and Stewart⁷⁹ and others¹⁰³ believe that the origin of the tumours is from alveolar epithelium. It is also the author's opinion that the tumours originate from alveolar lining cells. Light microscopic examination disclosed an intimate relationship of tumour cells to alveolar septa, and a resemblance between alveolar lining cells and neoplastic cells. Hyperplastic foci of alveolar cells were frequently present in the tumour bearing lungs. Recent electron microscopic studies confirmed the alveolar cell origin^{106,120} of chemically induced and spontaneous pulmonary adenomas. In ultrastructure studies, Svoboda¹²⁰ pointed out the resemblance of tumour cells to alveolar epithelium in terms of cell size, nuclear configuration, the presence of microvilli, the amount of distribution of endoplasmic reticulum, the size and structure of mitochondria, and more specifically, possession of lamellar-transformed mitochondria. Although structural similarity between alveolar lining cells and tumour cells does not necessarily imply identity, these morphological studies support the view that cells comprising pulmonary adenomas are derived from alveolar cells.

The cells undergoing neoplastic change and their modes of differentiation are of interest in the study of pulmonary carcinogenesis in mice. A striking contrast is present in the neoplastic proclivities, whether spontaneous or induced, of alveolar lining cells and epithelium of the bronchial tree in mice. Chemical carcinogens applied at sites remote from lungs by various methods have "increased the incidence of spontaneous adenomas" in mice and these have been said to arise from alveolar lining cells. ^{79,106,120}

But the same carcinogens impregnated in thread transfixed in lung ⁶⁶ or instilled in lung transplants ^{83,84,117,118} induced squamous cell carcinoma in bronchial epithelium. Epidermoid carcinoma has also been produced by direct application of a chemical carcinogen to the bronchial epithelium of the hamster. ⁷⁵

Although the question of whether alveolar lining cells are epithelial or mesenchymal is not resolved, squamous metaplasia might well be expected if alveolar lining cells were, in fact, epithelial cells. The possibility of a squamous metaplasia has been discussed in connection with studies of lungs in rats suffering from Vitamin A deficiency and from exposure to tars. ^{99,109} ⁸⁹ Lisco and ⁷¹ Gember and Watson described benign squamous metaplasia, together with carcinoma of alveolar lining cells in rats by radioactive materials. ^{117,118} Smith has described squamous metaplasia and epidermoid

carcinoma of alveolar lining cells in dibenzanthracene exposed lung transplants from foetal mice.

Chronic external gamma-radiation increased the incidence of adenomatous tumours that were morphologically identical to spontaneous pulmonary neoplasms in mice.^{91,92} On the other hand, alpha and beta internal emitters usually induced squamous cell carcinoma of the lung of mice, although a few undifferentiated carcinomas and adeno-carcinomas have been reported.⁷⁸ Continuous internal gamma emitters frequently produced bronchogenic squamous cell carcinoma and rarely adenomatous tumours.⁷⁸ Gates and Warren⁷⁷ induced bronchopulmonary epidermoid carcinomas in mice by an internal emitter of gamma radiation, cobalt 60; they regarded the source of the neoplasm as bronchial epithelium and found no evidence of neoplasms arising from alveolar cells.

Histological differentiations of benign from malignant pulmonary tumours in mice are disputable. Many investigators⁷³ 74,79,85,101,113 considered all of these tumours benign. Stewart¹¹⁹ expressed the opinion that all of these tumours malignant because of their local invasiveness, transplantability and ability to metastasize.⁹⁸ Magnus considered some of these tumours to be benign and others, that invaded the border of lung tissue, invaded lymphatics or metastasized, to be malignant. Biancifiori and Ribacchi⁶⁷ induced a total of 562 pulmonary tumours in mice and regarded 10 tumours as carcinoma on the grounds of atypical structure and local invasion of lung alveoli or bronchi. One of their animals

showed metastatic lesions. Slye et al¹¹⁶ found 163 mice with pulmonary tumours and of these malignancy was diagnosed in 63 (38.6%) mice. Magnus⁹⁸ reported a 75% malignancy rate.

Metastasis of spontaneous or chemically induced pulmonary tumours to remote sites is rare. No metastasis from pulmonary tumours in mice was observed in the present study. Sites of occasional metastasis reported in the literature are liver and ovary in one mouse,⁶⁷ mediastinum in two mice,¹¹⁶ chest wall, diaphragm or kidney in two mice,¹¹⁶ heart in one mouse,⁷⁰ kidney in one mouse,⁷⁰ mediastinum and kidney in one mouse,⁷⁰ liver in one mouse⁹⁸ and adrenal gland in one mouse.

Despite a lack of PAS positive substances in the tumours these lesions appear to be approximate counterparts of alveolar carcinoma or pulmonary adenomatosis in human and of Jaagsiekte of sheep. Bonne⁶⁹ noted a similarity between experimentally induced lung tumours in mice and certain phases of pulmonary adenomatosis (Jaagsiekte) in sheep. In respect to a similarity between pulmonary adenomatosis in man and in mice, two unusual cases (one an untreated virgin wild mouse and the other an untreated C₃Hb mouse) recorded by Horn et al⁸² are particularly interesting. Pulmonary tumours in these mice were strikingly similar to those in man, with respect to a single layer of columnar cells lining the alveoli. The cytoplasm of each lining cell contained a large globule of mucus and papillary tufts of proliferating

cells projected into the alveolar spaces in many places.

Possible causative agents of pulmonary tumours in mice have been suggested. Haaland⁸⁰ frequently found nematodes in the lungs of mice with multiple adenomata, and he suggested that the tumours were the end result of chronic irritation produced by these parasites. A viral aetiology of pulmonary tumours in mice was suggested first by Nettleship et al¹⁰⁵ who observed lesions composed primarily of mononuclear cells, with a few neutrophils. This suggested a virus pneumonia and was observed in large segments of lobes containing tumours. In studies of pulmonary tumours, Klärner and Gieseck^{86,87} described cytoplasmic inclusions containing 20 mu particles which they regarded as viral particles possibly aetiologically related to the tumours. However, Svoboda¹²⁰ found no evidence of virus or virus-like particles in any of the pulmonary tumours studied by an electron microscope.

SUMMARY AND CONCLUSIONS

The local application of minimal amounts of 20-methylcholanthrene dissolved in acetone to the uterine cervixes of two different strains of mice induced primary pulmonary tumours in 18 of 83 (22%) I.C.R. Swiss mice and in 1 of 80 (1.3%) phenotypically normal Pituitary Dwarf mice.

In 19 methylcholanthrene-treated mice with pulmonary tumours, carcinoma of the cervix and/or vagina occurred in only 3 mice, and epithelial dysplasia of the cervix and/or vagina occurred in only 2 mice.

Control I.C.R. Swiss mice or phenotypically normal Pituitary Dwarf strain mice treated with acetone or saline had no pulmonary tumours.

The tumour cells are regarded as originating from alveolar lining cells on the basis of light microscopic examination. Hyperplastic foci of alveolar cells in the lung were frequently found and morphological similarities between neoplastic cells and alveolar lining cells existed.

102 pulmonary tumours were classified histologically and 98 lesions (96%) fell into benign (adenoma) and 4 lesions (4%) into malignant (adenocarcinoma). Metastasis was not observed.

No specific secretory substances were detected in neoplastic cells by histochemical methods. Despite morphological similarities to human alveolar adenomatosis and Jaagsiekte of

sheep, pulmonary tumour cells in mice were virtually devoid of mucin.

Inflammation was so minimal that no significant correlation between inflammation and carcinogenesis of lung tumours in mice could be made.

Figures 14, 15 and 16

All sections are stained with haematoxylin, phloxine and saffron unless otherwise stated.

Figure 14. Multiple pulmonary tumours.

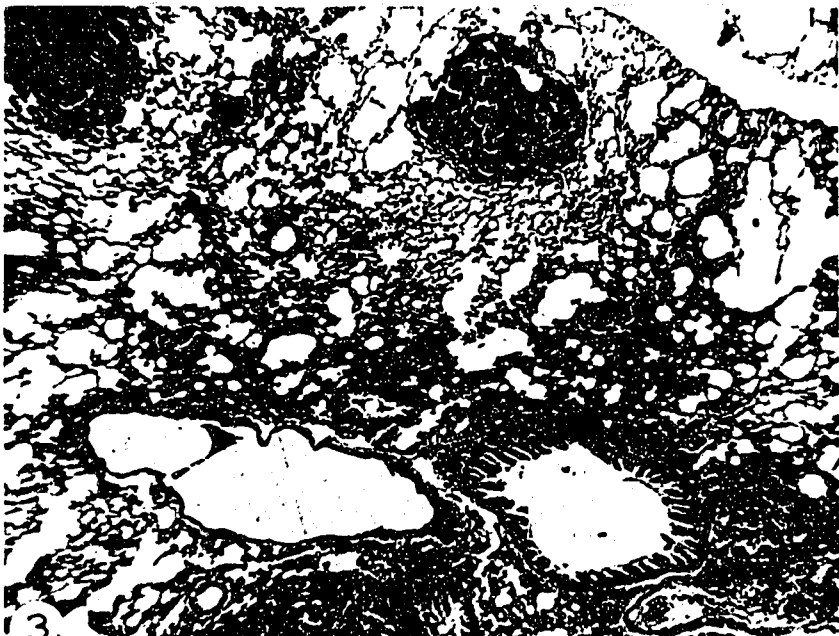
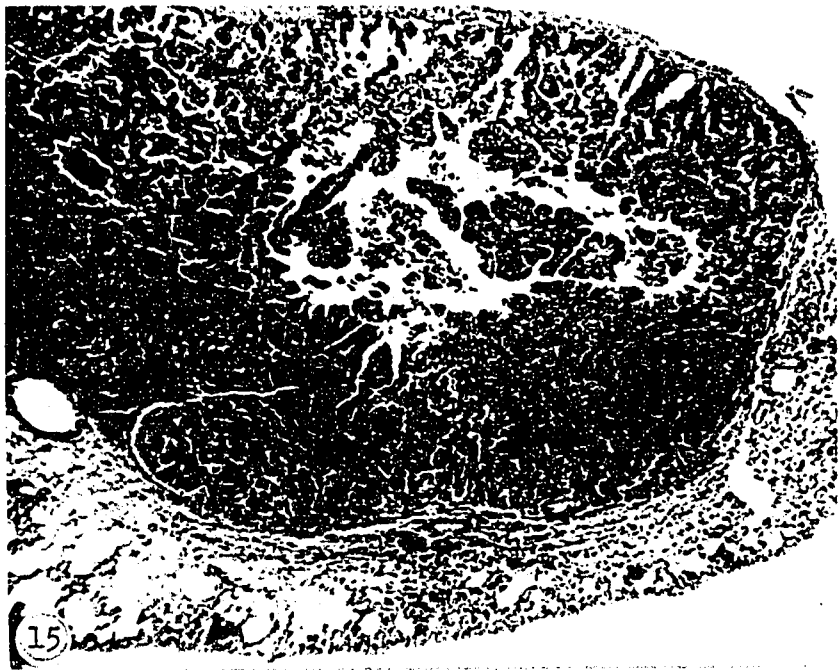
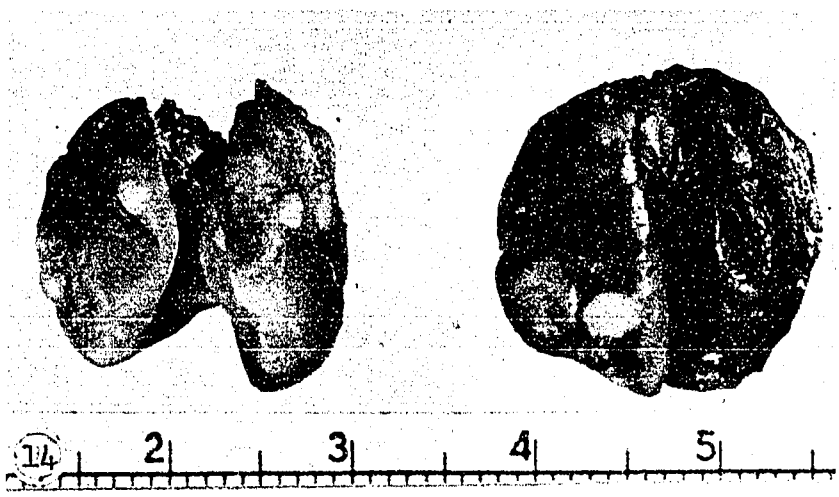
Experiment II. Lungs from two I.C.R. Swiss mice treated with 3 and 4 applications of methylcholanthrene respectively. Many bilateral, spherical nodules are seen. These are mostly peripheral in distribution.

Figure 15. Pulmonary adenoma.

Experiment III. Pituitary Dwarf strain mouse. The adenoma is subpleural, well circumscribed and surrounding alveoli are compressed. A papillary pattern predominates. X70.

Figure 16. Pulmonary adenomatosis.

Experiment III. The Pituitary Dwarf strain mouse had 8 pulmonary neoplasms bilateral in distribution. X32.



Figures 17, 18 and 19

Figure 17. Fibrosis in a pulmonary neoplasm.

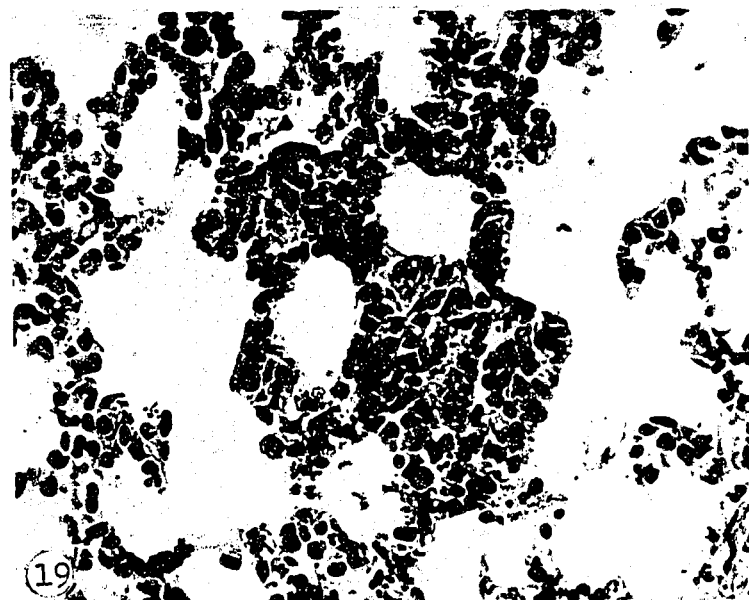
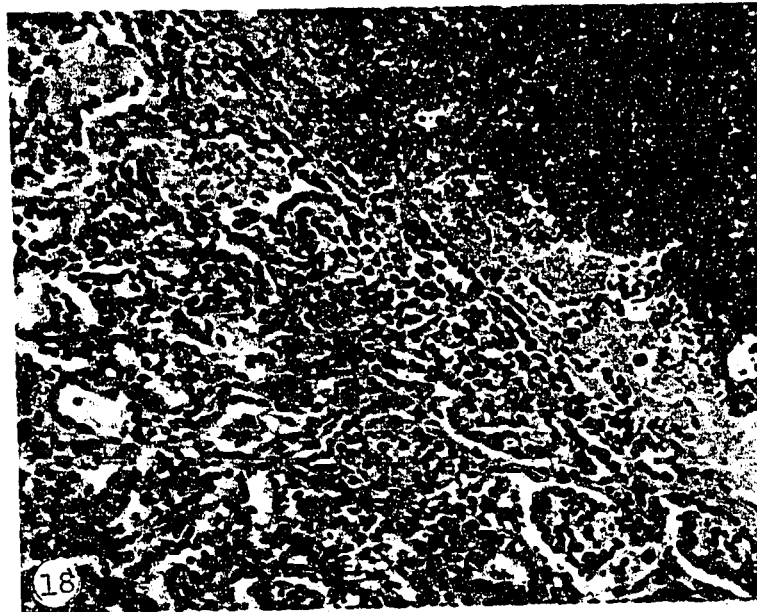
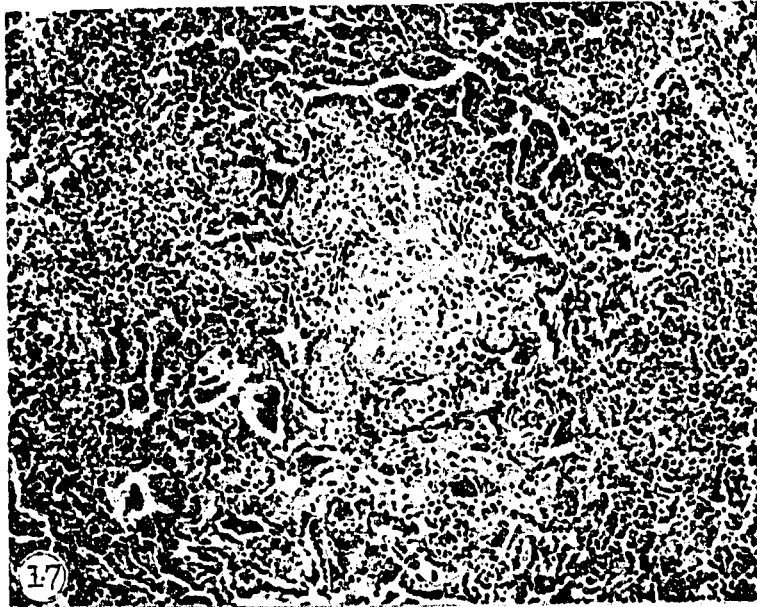
Experiment II. Methylcholanthrene-treated I.C.R. Swiss mouse. The centre of the neoplastic nodule shows considerable fibrosis. X110.

Figure 18. Inflammation in the vicinity of a tumour.

Experiment II. Note necrotic and inflammatory debris adjacent to an epithelial growth. X220.

Figure 19. Hyperplasia of alveolar lining cells.

Experiment II. I.C.R. Swiss mouse treated with methylcholanthrene. Multiple foci of hyperplastic alveolar lining cells in the tumour-bearing lungs. X180.



Figures 20, 21 and 22

Figure 20. Malignant change in adenoma.

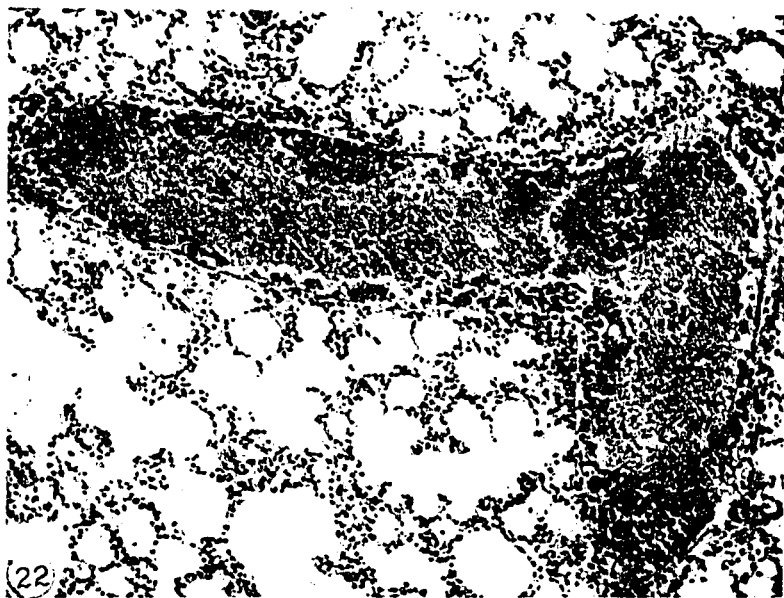
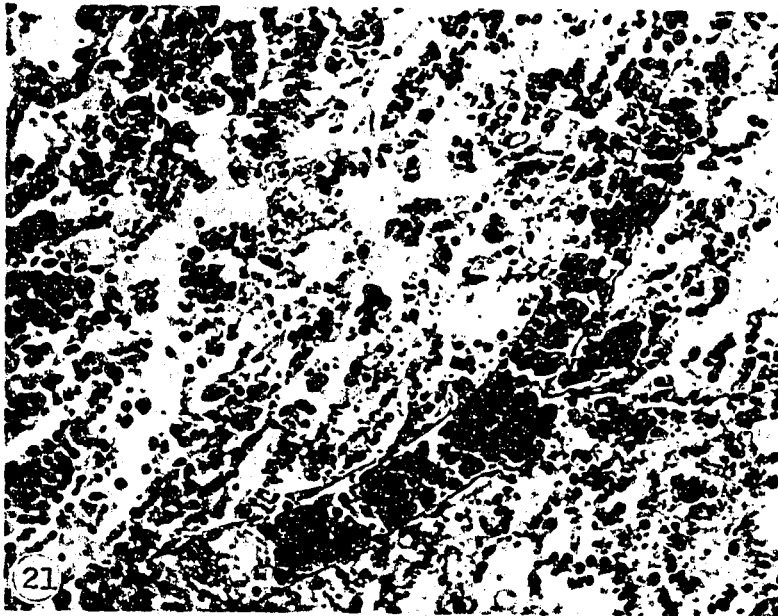
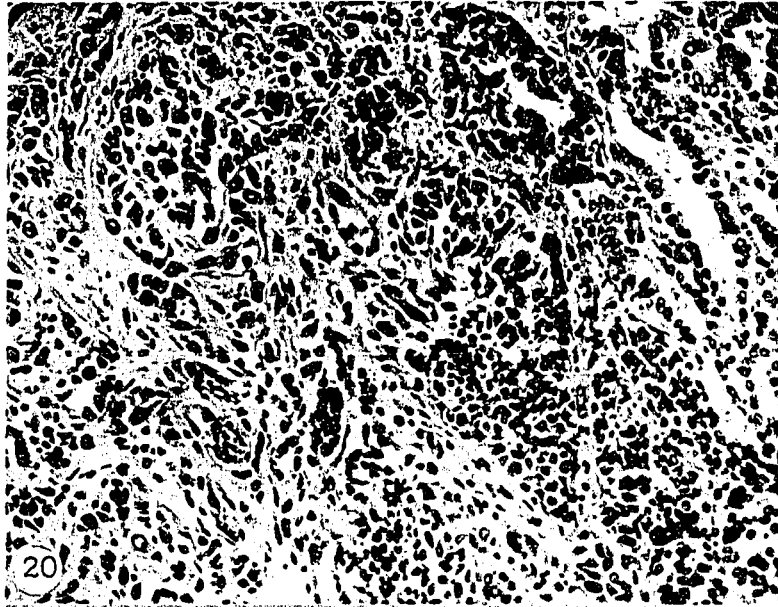
Experiment II. Malignant cells have pleomorphic, hyperchromatic nuclei and are arranged in disorderly fashion. An area interpreted as benign lies in the upper right corner of the illustration. X360.

Figure 21. Vascular involvement of tumour cells.

Experiment II. Neoplastic cells in a blood vessel near the periphery of a lesion. X220.

Figure 22. Tumour emboli in lung with multiple lesions.

Experiment II. Tumour cells are adherent to the blood vessel walls. X110.



Primary Mammary Tumours in Mice
Following the Application of
20-Methylcholanthrene to the
Uterine Cervix.

INTRODUCTION

Mammary tumours of the mouse have been studied in detail by many workers in the past particularly with respect to the genetics, virology, endocrinology and biology of these tumours.

This chapter deals with a presentation of seven cases of mammary cancer in methylcholanthrene-treated mice and one case in an acetone-treated mouse. The carcinomas developed only some time after minimal dose applications of the substances to the uterine cervix..

REVIEW OF THE LITERATURE

Tumours of mammary tissues and lungs are the commonest spontaneous neoplasms in mice. Crisp (1854)¹²⁹ is credited with the first description of a mammary tumour in the mouse. Livingood¹⁴¹ in 1896 reported spontaneous tumours encountered in the mammary regions of mice, and suggested their origin from the mammary gland. After the publication of Jensen's paper and following the wide distribution¹³⁶ of his tumour to other cancer laboratories, these tumours of the mouse became a standard tool for a variety of investigations.

The incidence of spontaneous mammary cancer may vary between more than 90% (C₃H) and less than 1% (C₅₇Black) in different strains of mice.¹³² The highest incidence of mammary tumours found is 93.2% in breeding females of BALB/cf mice.¹⁵⁴ Spontaneous mammary cancer in the mouse is confined to the female sex.¹³² Breeding females show a high incidence of mammary tumours and the incidence increases with the number of litters born, but virgin females develop cancer rarely and then much later in life.¹³⁷

For nearly three decades it has been known that the administration of certain chemical carcinogens induces mammary cancer in mice and rats. Mammary tumours have been induced by skin painting,^{123,130,142,143} subcutaneous implantation,¹²⁶ intravenous injection,^{131,150} oral administration,^{124,134,135,155} and other routes.^{134,149}

142
Haisin and Coolen in 1936 repeatedly painted the skin of mice with 20-methylcholanthrene or with benzo(a)pyrene and observed that, in addition to cancer of the skin, mammary cancer arose in 18% of the mice.
130
Engelbreth-Holm painted mice and found that mammary cancer arose only in females. This experiment demonstrated the sex difference in the process. 123
Andervant and Dunn obtained mammary cancer in milk-agent-free female mice by percutaneous applications of methylcholanthrene at different sites. The induction of breast tumours by cutaneous application of methylcholanthrene has been studied in mice of several strains by Marchant. 143

128
Bonsor in 1954 implanted paraffin wax pellets containing a small dose of the 20-methylcholanthrene into the breast tissue and 16 weeks later mammary carcinomas had occurred in little more than half of the IF strain of mice.

155
Wilson, DeLds and Cox were the first to observe that distant tumours arose after oral carcinogen administrations. In their experiments, H-2-fluorenyl-acetamide evoked tumours of liver, bladder and mammary gland in rats. When milk-agent-free C₃H virgin mice (C₃Hb) were given a limited dose, by oral administration, of four chemical carcinogens: 9,10-dimethyl-1,2-benzanthracene (DMBA), 20-methylcholanthrene (MC), 1:2:5,6-dibenzanthracene (DBA) and 3,4-benzopyrene (BP); mammary tumours were induced in

the percentage of 52.9 (DIBBA), 31.4 (HC), 13.8 (DBA), and 3.6 (BP) respectively.¹²⁴

Feeding of a single dose of polycyclic hydrocarbons sufficed to induce mammary cancer in rats.^{134,135}

In Sprague Dawley adult female rats, mammary cancer developed invariably after oral administration of a single 20 mg. dose of 7, 12-dimethyl-benz(a)anthracene, dissolved in sesame oil.¹³⁴ The earliest mammary cancer induced by this technique was detected by palpation 20 days after the administration, and all rats had mammary cancer within a few weeks thereafter.

Implantation of a compressed pellet of 20-methylcholanthrene in the spleen was followed by the development of mammary cancer.^{134 149} Orr in 1951 was able to induce mammary tumours by intranasal administration of 20-methylcholanthrene.

MATERIALS AND METHODS

A total of 269 mice in Experiments I, II, III and V were studied. 20-methylcholanthrene dissolved in acetone was applied to the uterine cervixes of test mice. Control mice were treated similarly with acetone or saline alone. Methods of carcinogen applications are described in detail on page 10 to 16.

For histologic examination, tumour tissues were fixed in 10% formalin, embedded in paraffin, cut three microns in thickness and stained routinely with haematoxylin, phloxine and saffron dyes.

OBSERVATIONS

Five of 60 (8.3%) I.C.R. Swiss mice and 2 of 52 (0.4%) Pituitary Dwarf strain mice treated with 4 or more methylcholanthrene applications developed single or multiple mammary gland carcinomas. One of 68 (1.5%) acetone-treated I.C.R. Swiss mice developed a similar lesion. The first tumour appeared on the 65th day after methylcholanthrene application. The afflicted mice died from 21 to 93 days after their mammary cancer first appeared. The average survival time after the tumour discovery was 43 days. Three mice had multiple lesions (2-4) and 5 mice had a single tumour.

The neoplastic masses (Figure 23) appeared subcutaneously along the mammary line and were discovered first as small firm movable ovoid or spherical but coarse nodules. They grew rapidly and when the mass became large in size, the overlying skin surface became necrotic and ulcerated. The masses were well circumscribed and were easily separated from the surrounding structures. The average size of the tumours at the time of postmortem examination was 2.8 cm in diameter and the largest being 4.3 cm. On cut surface, the tumour tissue was usually grey-white and soft, often with many areas of haemorrhages and necrosis (Figure 24).

The histologic differentiation of 13 neoplastic lesions from 8 mice was variable but all were malignant

and adenocarcinoma in type. There was a varying degree of lumen formation, and neoplastic epithelial cell lining ranged from one to several layers in thickness (Figure 25). These cells had frequent mitoses, some of which were atypical. Abortive gland-like spaces were separated by thin septa of stroma with large blood vessels. Cells were cuboidal or low columnar with oval or round hyperchromatic nuclei. The cytoplasm was abundant and showed marked pleomorphism.

The appearance of epithelial pearls and cornified or non-cornified squamous epithelial cells (Figure 26) was observed in 3 out of 13 tumours. Areas of cellular overgrowth in papillary fashions were also occasionally seen.

Two I.C.R. Swiss mice had pulmonary metastases and one also had mediastinal lymph node metastasis (Figure 27). Interestingly only one mouse had carcinoma in the cervix or vagina. One mouse had an area of dysplasia in the vaginal vault and one had epidermoid carcinoma of the upper vagina. None of the mice with mammary carcinomas had lesions in the ovary or other endocrine organs.

DISCUSSION

The development of mammary cancer in mice depends on several factors: 1) hormonal stimulation, 2) the milk agent or mammary tumour agent (MMA), 3) genetic constitution and 4) other environmental factors such as diet.

Direct evidence for the hormonal influence has been obtained in several ways by many investigators. Early ovariectomy inhibits or greatly delays mammary tumour formation in females. ^{140;146} Mammary cancer develops in some castrated male carrying ovarian transplants. ¹⁴⁷ Mammary tumours have been induced in male mice of many strains by the administration of synthetic oestrogens. ¹⁴⁴ Mammary carcinomas have been induced by methylcholanthrene in male mice ¹⁵¹ that have been given oestrogen.

¹⁵² Shinkin pointed out the parallelism between the incidence of mammary tumours in breeding females of various inbred strains and the readiness with which carcinomas of the breast were induced by oestrogens in male mice. He suggested that the oestrogens were accelerators in the females and evocators in the males.

By reciprocal crosses between females of a high and males of a low mammary tumour strain, and vice versa, the tumour incidence in the hybrid young was found to resemble more or less that of the mother's strain. ¹⁴⁸ These results could not be explained on principles of Mendelian inheritance and pointed to some extra-chromosomal influence.

The critical experiments to explain this phenomenon were conducted by Bittner¹²⁵ and Bittner and Little¹²⁶. If the babies from mothers of a high mammary tumour strain were removed immediately after birth, and allowed to be foster-nursed by mothers of a low tumour strain, the tumour incidence in the fostered young was much reduced. Conversely, the young of low-tumour strain suckled by high-tumour foster mothers acquired a high tumour susceptibility. The extra-chromosomal factor was evidently something transmitted from mother to young through the milk. Extensive subsequent investigations have shown that this milk agent has many of the properties of a virus.¹²⁷

Although the development of mammary tumours in the mouse is largely dependent on hormonal stimulation and the milk influence, genetical susceptibility is of concern and acts through several channels. The effect of the milk agent depends on the strain in which it occurs. Genes also control the hormonal aspects of mammary tumour development.¹³³

Various environmental factors affect the genesis of mammary tumours in mice.¹²⁷ Restricted feeding or dietary deficiencies reduce the incidence of mammary tumours.¹²⁷ Diet may exert an effect by influencing the growth of the body or of the breast, or by modifying the amounts of and relationship between various endocrine secretions. It is not known whether diet or other environmental components influence the transmission and otherwise modify the action

of the milk agent.

As noted in the review of literature, carcinogens can induce mammary carcinomas both by direct and remote applications, but the precise mechanism of carcinogenesis is not clear.

¹³⁸
Khanolkar in 1961 attempted to study mechanism of chemical induction of breast cancer in mice after administration of carcinogens by different routes. 20-methylcholanthrene was administered by three methods: skin painting, subcutaneous implantation of carcinogen pellets, and painting the ovary. Two probable pathways for the action of methylcholanthrene were suggested. One acted directly on the epithelium of the mammary gland and initiated the neoplastic process. The other was through the endocrine tissue of the ovary, which effected certain proximate changes in the ovarian tissue and remote changes in the breast parenchyma. The cells of the breast parenchyma were thus transformed into potential neoplastic cells.

¹³⁹
Kirschbaum et al compared the histogenesis and histological features of methylcholanthrene induced mammary cancer and spontaneous mammary cancer in the mouse. The major difference was the prevalence of squamous metaplasia of ductal epithelium during the development of the carcinogen-induced tumour. Many workers have noted squamous metaplasia in mammary tumours induced by carcinogenic hydrocarbons.^{123,149} Of 45 tumours from the strain of NIH mice, Strong

and Williams¹⁵³ found eleven were of the squamous metaplasia type with keratinization. Bonser¹²⁸ studied early changes antecedent to tumour formation evoked by chemical carcinogen applications, and rarely found squamous metaplasia. Squamous metaplasia was seen in 3 of 13 mammary carcinomas in the present experimental work.

Orr¹⁴⁹ listed squamous metaplasia and other histologic points of difference between spontaneous and methylcholanthrene induced tumours. In carcinogen-induced tumour, the tubules are larger and less regular in size, there are solid acinar and trabecular structures with intervening fibrous or fibroblastic stroma, there is a high degree of cellular pleomorphism, the stroma is in general much more prominent than in the spontaneous tumours, and a high proportion of the tumours are spindle cell sarcomata or carcinosarcomata.

As the methylcholanthrene-induced tumours differ in certain respects from spontaneous murine tumours of mammary glands, the possibility must be considered that the processes are unrelated, and that the carcinogen does not augment a spontaneous mechanism.

The significance of experimental data in mice, in relation to the human disease, is not easy to assess. The following are some comparative parts of interests.

- (a) Although cancer of the breast in women appears to be influenced by heredity, the in-

fluence is slight.

- (b) There is, as yet, no convincing evidence that prolonged oestrogen administration is carcinogenic for the breast in humans. In mice mammary tumours develop more frequently in breeders than in virgins, while in women the opposite seems to be the case. This fact has been attributed to the fact that the mouse corpus luteum does not produce progesterone¹⁴⁵ in the virgin, while in women it does.
- (c) The possibility that a tumour agent may exist in human milk by which mammary cancer is transmitted from the mother to female offspring has been seriously considered. Animal experiments indicates, however, that such an agent exists only in the mouse.

SUMMARY AND CONCLUSIONS

Mammary carcinomas developed in 5 of 60 I.C.R. Swiss mice and 2 of 52 Pituitary Dwarf strain mice that were given 0.4 ml. or more of 0.5% 20-methylcholanthrene applications to the uterine cervix. One of 68 acetone-treated mice in the control groups showed similar mammary neoplasms.

The mammary neoplasms encountered were principally adenocarcinomatous pattern with variant microscopic features. Three of 13 tumours were adenoacanthomas. Two mice bearing mammary carcinomas had pulmonary metastases and one of these also had mediastinal lymph node metastasis.

The first tumour appeared on the 65th day after methylcholanthrene application and the afflicted mice died from 21 to 93 days after their mammary cancer first appeared.

Development of mammary tumours in mice depends upon several factors including hormonal stimulation, the milk or virus agent, genetic constitution, diet and environmental factors.

Carcinogenic hydrocarbons induce mammary cancer in milk-agent-free mice of strains which are either susceptible or resistant to the development of spontaneous mammary carcinoma.

Figures 23,24 and 25

All sections are stained with haematoxylin, phloxine and saffron unless otherwise stated.

Figure 23. Carcinoma of mammary gland.

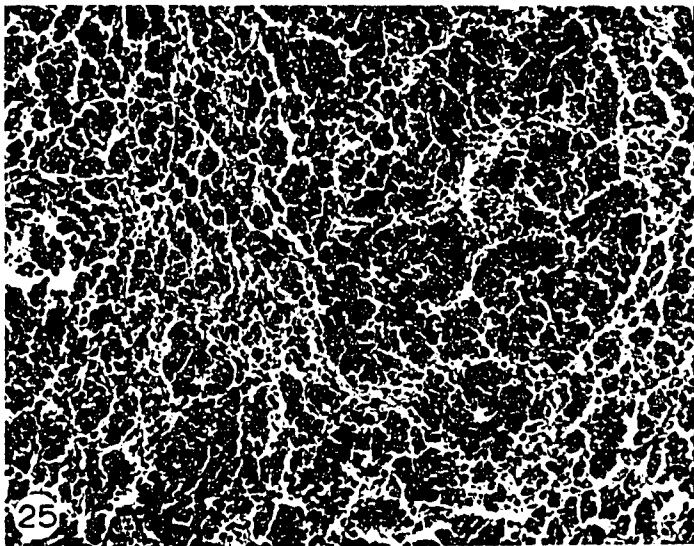
Experiment II. I.C.R. Swiss mouse given 11 methylcholanthrene applications to cervix uteri. The mass appeared on the 65th day of the experiment. This photograph was taken on the 108th day of the experiment.

Figure 24. Bisected mammary tumour.

An ovoid grey-white soft mass with areas of haemorrhage and necrosis.

Figure 25. Adenocarcinoma of mammary gland.

Experiment V group B. This methylcholanthrene-treated Pituitary Dwarf strain mouse lived 160 days. The lesion is highly anaplastic. X70.



Figures 26 and 27.

Figure 26. Adenoacanthoma of mammary tissue.

Experiment V group B. One of the variant microscopic features of the tumour shown in Figure 25. Note areas of squamoid differentiation of neoplastic cells. X225.

Figure 27. Pulmonary metastasis from adenocarcinoma of mammary gland.

Experiment II. I.C.R. Swiss mouse given 4 methylcholanthrene applications. Most of the metastatic nodules lie in peribronchial lymphatics. X70.



Figures 28, 29 and 30.

Figure 28. Vasculitis in cervix uteri.

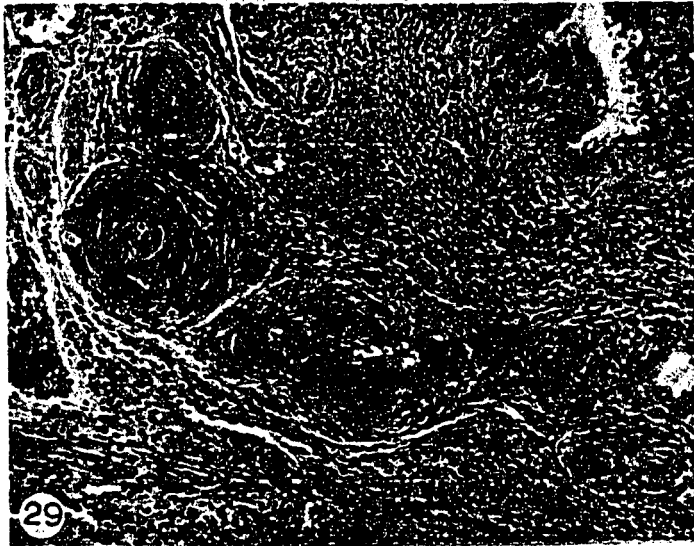
Experiment I. This vessel, presumably a precapillary arteriole, shows fibrinoid change in its wall and perivascular infiltration of lymphocytes. Basal cell hyperplasia of cervical epithelium is seen in the upper part of the field. X115.

Figure 29. Vasculitis in cervix uteri.

Experiment V group A. Both venules and arterioles had markedly thickened walls with necrosis and perivascular round cell infiltration. X115.

Figure 30. Vasculitis in parametrium.

Experiment V group B. Pre-capillary arterioles showing fibrinoid change with mural thickening, narrowing of lumina and perivascular leucocytic infiltrations. X185.



Figures 31, 32 and 33.

Figure 31. Vasculitis in spleen.

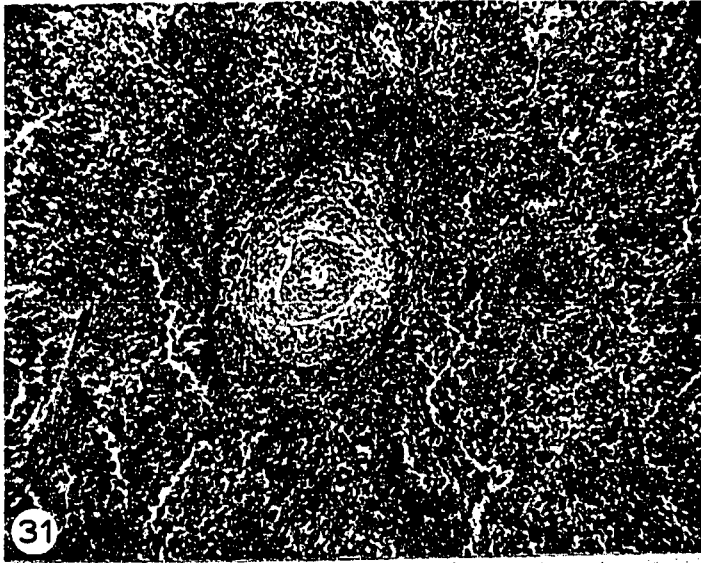
Experiment V group A. Methylcholanthrene-treated Pituitary Dwarf strain mouse. Note thickened fibrosed wall of arteriole. X74.

Figure 32. Vasculitis in lung.

Experiment V group A. Acetone-treated Pituitary Dwarf strain mouse. Note extremely thickened walls of arteries with marked subintimal fibrous hyperplasia and partially occluded lumina. X110.

Figure 33. Necrotizing vasculitis in kidney.

Experiment V group A. Walls of large and small arteries as well as arterioles show fibrinoid changes. Note a glomerular tuft at the hilus with thickened necrotic walls. X225. Alcian Blue - PAS stain.



Figures 34, 35, 36 and 37.

Figure 34. Amyloidosis of spleen.

Experiment I. Methylcholanthrene-treated I.C.R. Swiss mouse. Note pale homogenous amyloid deposition in the form of crescentic bands. X35. Congo Red stain.

Figure 35. Amyloidosis of kidney.

Experiment III. Acetone-treated Pituitary Dwarf strain mouse. Amyloid deposited in glomerular tuft. X450.

Figure 36. Amyloidosis of liver.

Experiment II. Methylcholanthrene-treated I.C.R. Swiss mouse. Amyloid material is deposited in sinusoids and hepatic cords are atrophic. X225.

Figure 37. Plasma cells and amyloid deposits in spleen.

Experiment II. I.C.R. Swiss mouse treated by 9 methylcholanthrene applications. The deposit of amyloid is at the right of picture. Proliferation of plasma cells about amyloid deposits was often conspicuous. X225.



Reticuloendothelial Cell Hyperplasias
and Crystalline Material in
Laboratory Mice.

INTRODUCTION

The remarkable activity of the reticuloendothelial system in disease results in a variety of anatomic changes. Hyperplasia of reticuloendothelial cells, presumably histiocytes, was observed in various organs of mice in these studies. These cells had a peculiarly streaked acidophilic cytoplasm and many contained crystalloid material. Crystalloid material was also observed extracellularly.

Similar crystals and reticuloendothelial cells have been described, ^{161,164,166,167,176} yet their true identity and significance has not been completely established. The purpose of this chapter is to investigate the nature and significance of the lesions that were characterized by collections of acidophilic cells and crystalloid material in laboratory mice.

REVIEW OF THE LITERATURE

Mice

Crystalloid materials in mice were noted by Haaland¹⁶⁶ in 1905 and Tyzzer¹⁷⁶ in 1909 described and photographed crystalloid bodies that were present in the lungs of mice. Green¹⁶⁴ noted both extracellular and intracellular crystals in lungs, and in three isolated cases in the acinar cells of the pancreas, in the lumina of the endometrial glands, and in the tissue of a sarcoma of the thigh. His histochemical studies indicated that the crystalline substance was protein.

In Dunn's monograph¹⁶¹ there is a photomicrograph illustrating large mononuclear cells with peculiarly streaked eosinophilic cytoplasm in a lymph node from a leukemic mouse.

Horn et al¹⁶⁷ reported two unusual cases of mice with multiple pulmonary adenomatosis characterized by mucus secreting columnar epithelium. In the pneumonic lesion in one of the mice, there was an infiltration with mononuclear leucocytes. The cytoplasm of these cells were stained with eosin and contained a few pigment granules and innumerable small needle-shaped crystals. These crystals were black with iron haematoxylin, faint pink with fuchsin, dark brown with phosphotungstic acid haematoxylin, brown with silver, and pale blue with toluidine blue. Horn did not suggest

what these staining reactions indicated.

Man

Similar crystals and reticuloendothelial cell hyperplasia have been observed in man.

Crystals contained within nodules of multiple myeloma were described in the ribs, vertebrae, sternum, lymph nodes and testes by Glaus.¹⁶² Abrikossoff and Wulff¹⁵⁶ reported a case of multiple myeloma in which needle-like, rod-like and rectangular crystals were found in the periphery of a tumour nodule in the rib. These authors showed by chemical analysis that the crystals were closely related to Bence Jones protein.

Ritchie and Meyer¹⁷⁴ in 1936 reported the case of a 36 year old white female, with reticuloendothelial hyperplasia involving the liver, spleen, lymph nodes, bone marrow and the connective tissue. This was accompanied by formation of bizarre giant cells and crystals of unknown composition in phagocytes in widely distributed regions. Crystals within the cytoplasm of phagocytes were stained strongly by eosin, basic and acid fuchsin, and trinitrophenol (Van Gieson's stain). They were unstained by congo red and were not metachromatic with methyl violet. Acids and alkalis of moderate strength did not dissolve them, nor did fat solvents. They considered that this case was one of aleukemic reticuloendotheliosis.

A case of purpura haemorrhagica accompanied by widespread occurrence of crystals in the cytoplasm of phagocytes was noted by Agress and Smith.¹⁵⁷ On post mortem examination, many phagocytic cells containing cytoplasmic crystalline bodies were seen in tissues such as the spleen, marrow of femur, ribs and sternum and renal tubules, adrenal cortex, liver, lungs and heart. With haematoxylin and eosin, crystals were stained pink, with the Dominici stain deep blue, with the Unna-Pappenheim stain bright deep red, with Masson's polychrome stain bright red and with Foote's modification of the Bielschowsky stain pinkish-brown. These authors suggested that the crystalline material was protein in nature.

Various other unknown crystalline materials have been observed by several authors.^{160,168,171}

Intranuclear Crystalline Material in Other Animals

Similar acidophilic crystalline materials contained in nuclei of epithelial cells of the liver and kidneys of dogs,^{158,169,177} and in the hepatic cells of wolves, foxes and jackals¹⁷⁸ have been observed and reported in the literature.

In 1909, Brandts¹⁵⁸ reported square crystalline acidophilic, iron-negative intranuclear inclusions in the hepatic epithelium of a dog. He expressed doubt as to their identification as true haemoglobin crystals. Nicolau and Kopciowska¹⁶⁹ in 1936 also reported acidophilic cryst-

alline intranuclear inclusions in the livers of 27 of 44 dogs and in kidneys of 12 of 27 dogs with inclusions in the liver. These inclusions were non-birefrigent and were demonstrated to be iron-free on microincineration. They postulated that such crystalline inclusions were caused by a saprophytic virus. Thompson et al¹⁷⁷ in 1959 observed acidophilic crystals in epithelial cells of the liver and kidneys of 13 dogs. Their histochemical and physical procedures carried out upon paraffin-embedded tissue sections showed that these crystals were not composed of haemoglobin or any of its iron-containing derivatives, nor of minerals, lipids, deoxyribonucleic acid, ribonucleic acid, glycoproteins, cholesterol, glycogen, mucin, mucopolysaccharides, polysaccharides or glycolipids. They have considered the possible role of altered protein metabolism within affected nuclei.

MATERIALS AND METHODS

Autopsy tissues of internal organs and lymph nodes of various regions of a total of 269 mice were studied for acidophilic crystals and reticuloendothelial cell hyperplasia. 118 phenotypically normal Pituitary Dwarf strain and 151 I.C.R. Swiss female mice were examined.

In order to detect crystalloid material or reticuloendothelial cell hyperplasia in these animals, complete histologic examinations were done on the following tissues: brain, sternal, vertebral and femoral bone marrows, lymph nodes from various regions, thymus, heart, lungs, kidneys, spleen, adrenals, uterus, ovaries, vagina, liver, skin, stomach and intestine.

Formalin fixed tissues were embedded in paraffin, cut three micra in thickness and stained routinely with haematoxylin, phloxine and saffron. Sections of tissues with reticuloendothelial cell hyperplasia and crystalloid material were stained by a variety of techniques: alcian blue, ¹⁷³amidoblack for protein and benzidine for peroxidase, ¹⁷²congo red, Gmelin's reaction ¹⁵⁹for haematoidin, Gomori's iron reaction, ¹⁶⁵haematoxylin, phloxine and saffron(HPS), ¹⁷⁰haematoxylin and eosin, methyl green-pyronin, ¹⁶⁵methyl violet, ¹⁶⁵periodic acid-Schiff reaction, ¹⁷²Mayer's mucicarmine, ¹⁶⁵methylene blue, ¹⁶⁵phosphotungstic acid haematoxylin (Mallory's PT&H), ¹⁶⁵von Kossa's stain for calcium and toluidine blue dyes.

Frozen sections of lungs were stained with sudan III and oil red O for the demonstration of lipids, and with Schultz method¹⁷² for cholesterol and cholesterol esters.

Sections of fresh and 10% formalin fixed tissues, both unstained and stained, were examined under polarized light and by fluorescent microscope. The light source was the Leitz fluorescence equipment which has an Osram HB 200 maximum pressure mercury vapor lamp, with U.V. filter 2 mm UG 1 and 4 mm UG1 for U.V. light fluorescence and Blue Filter BG 12 for blue light fluorescence.

OBSERVATIONS

Five I.C.R. Swiss mice (3 methylcholanthrene-treated and 2 acetone-treated) had localized collections of reticuloendothelial cells, presumably histiocytes, in the lung and pulmonary hilar lymph node. Two of these mice also had intra and extracellular crystalloid materials in the lung, gall bladder and pulmonary hilar lymph node.

Case 1

The lungs of an acetone-treated I.C.R. Swiss mouse that lived 250 days were grossly firm, grey-brown to yellow, and the cut surfaces contained fine nodularities which were characteristically grey-white in the center with surrounding yellowish zones (Figure 38). Microscopically, the grey-white tiny nodules were seen to be extensive lymphocytic and other mononuclear cell infiltrations in bronchial and peribronchial walls. There were proliferations of bronchial epithelium. The remaining lung parenchyma was completely replaced by acidophilic peculiarly streaked histiocytes (Figures 39 and 40). Involved were the left lung and the middle and inferior median lobes of right lung. A further interesting observation was the presence of various shaped acidophilic crystalloid materials scattered extracellularly and intracellularly throughout bronchial lumina and peribronchial spaces. Occasionally this material was contained in histiocytes. The cytoplasm of bronchial and bronchiolar

epithelium was in areas quite as acidophilic as the histiocytic cytoplasm and the crystalloid materials. These epithelial cells were almost twice as large as other normal epithelial cells and gave completely different staining reactions. These reactions are described in detail on page 131.

The gall bladder was the only other organ in which the crystals were seen. This organ was normal in size and grossly unremarkable. Microscopically, however, acidophilic crystals and acidophilia of epithelial cytoplasm was striking (Figure 41). In the gall bladder there were minimal chronic inflammatory changes but no collections of histiocytes.

Case 2

An acetone-treated I.C.R. Swiss mouse, that lived 220 days had gross and microscopic pulmonary changes similar to those of Case 1. Whole lobes of the right and left lungs, except the right inferior lateral lobe, showed complete replacement of air sacs by histiocyte collections and peribronchial and bronchial lymphocytic and plasmacytic infiltrations (Figure 39). Some of the bronchial lumina were dilated and filled with mixtures of lymphocytes, histiocytes, a few granulocytes and many crystals. The crystalloid substance was the same kind of material as described in Case 1, but in this case there were fewer crystals in the lungs. Areas of bronchial and bronchiolar epithelium showed the

same morphologic and histochemical abnormality as described in Case 1.

One of the pulmonary hilar lymph nodes had occasional acidophilic, striated histiocytes and extracellular, polygonal small crystals. Other organs or sites were not similarly involved.

Case 3

In a 20-methylcholanthrene-treated I.C.R. Swiss mouse that lived 343 days, both lungs had a firm, grey-pink pleural surface and on cut surfaces, tiny grey-yellow nodularities were seen. Microscopically, there was a chronic bronchitis, peribronchitis and pneumonitis. There was complete replacement of air sacs by histiocytes in the inferior median and lateral lobes of the right lung. The other lobes of the right lung had scattered collections of such histiocytes. No crystals were found in the affected lung, and no other organs were involved.

Case 4

A 20-methylcholanthrene-treated I.C.R. Swiss mouse that lived 452 days after the application had changes of pseudoepitheliomatous hyperplasia, and poorly differentiated epidermoid carcinoma of the cervix uteri with extension to the vaginal vault and an epidermoid cyst of the vulva. The lungs contained multiple pulmonary adenomatoses, 27 adenomas in the right lung and 8 in the left lung. Microscopically,

there were marked histiocyte infiltration in alveolar sacs and septa in the left lung. No crystals were observed in the lungs and no histiocytic lesions were found in other organs.

Case 5

A 20-methylcholanthrene-treated I.C.R. Swiss mouse that lived 135 days had mammary carcinomas in the left and right subauricular regions, with metastases to both lungs. The right inferior median lobe, which was spared from metastatic neoplasm, had extensive histiocyte infiltrations, and minimal infiltrates of lymphocytes and plasma cell especially around the bronchial tree. The other lobes of the lungs and other organs were uninvolved.

Histiocytes

The histiocytes were large and had single or multiple nuclei. The cytoplasm was characteristic, being markedly acidophilic and containing peculiar fine needle-shaped streaks which were parallel to each other (Figure 40). The cells were round and ranged from 10 to 30 u, most cells being 8 to 20 u, in diameter. When nuclei were multiple, they were congregated, conglomerated and eccentric. The largest number of nuclei encountered in one cell was 22.

The results of various staining methods are listed in Table 4. The cytoplasm of this type of cell was stained pink-red by pyronin, deeply red by phloxine, pink-red by eosin, blue by toluidine blue, violet by methyl violet

(not metachromatic), and faintly yellow by congo red. Histiocytes were positive for Gomori's iron reaction (Figure 45), and slightly positive to the PAS staining technique. With amidoblack about one-third of the cells stained light blue. This stain gave a similar reaction with haemoglobin and elastic tissues.

Negative staining results were obtained by sudan III, oil red O for fat, von Kossa's stain for calcium and minerals, alcian blue, benzidine, Mayer's mucicarmin for mucin, Gmelin's reaction for haematoidin, methylene blue and Schultz method for cholesterol and cholesterol esters.

Some of the histiocytes which were not so deeply acidophilic appeared as "foam cells" but contained several sharp needle-shaped striations. Cytoplasmic striations were well demonstrated by PTM (Figure 40) but were only faintly stained by the other methods.

Crystalloid Materials

Crystalloid materials were of various shapes and sizes. The crystals were needle-shaped, rod-like, rectangular, or polygonal (Figure 42). The size of crystals varied greatly and measured from 10 to 127 μ in length and 1 to 55 μ in width. The commonest size of intracellular crystals was about 10 μ in length and 2 to 3 μ in width, that of extracellular crystals was about 35 μ in width. The crystals were colourless, but stained by various dyes.

The results of the various staining methods are listed in Table 4. Crystals were phloxinophilic, eosinophilic, pyroninophilic, violet by methyl violet, blue by toluidine blue and slightly yellow by congo red dyes. About two-thirds of the crystals were stained by amidoblack.

Negative staining results were obtained with alcian blue, benzidine, Gomori's reaction for iron, PAS, mucicarmine, sudan III, oil red O, Gmelin's reaction for haematoidin, methylene blue, von Kossa's for calcium and Schultz method for cholesterol and cholesterol esters.

Abnormal bronchiolar, bronchial or gall bladder epithelial cells gave the same reaction as the crystals, but differed in reaction from histiocytes and normal epithelial cells. Normal epithelial cells were positive by PAS, alcian blue and mucicarmine and were negative by amidoblack or benzidine staining methods. The abnormal epithelial cells stained negatively by PAS, alcian blue, benzidine and mucicarmine, and positively by the amidoblack method.

The crystals, cytoplasm of histiocytes, and abnormal epithelial cells showed no birefringence under the polarized light and were faintly fluorescent by U.V. and blue light fluorescence microscopy.

Table 4. Summary of Histochemical Studies of Histiocytes and Crystals.

Staining Methods	Cytoplasm of Histiocytes	Crystals
Alcian blue	Negative for mucin	Negative for mucin
Amidoblack	About 1/3 - light blue	About 2/3 - light to mod. blue
Benzidine	Negative	Negative
Congo red	Light yellow	light yellow
Gmelin's reaction for haematoidin	Negative	Negative
Gomori's iron reaction	Positive	Negative
Haematoxylin and eosin	Eosinophilic	Eosinophilic
Haematoxylin, phloxine and saffron	Phloxinophilic	Phloxinophilic
Mallory's PTAH	Clear to light brown (streaks demonstrated clearly)	Clear to light brown
Mayer's mucicarmine for mucin	Negative	Negative
Methyl-green pyronin	Pyroninophilic	Pyroninophilic
Methylene blue	Negative	Negative
Methyl violet	Blue violet (not metachromatic)	Blue violet (not metachromatic)
Oil Red O for fat	Negative	Negative
Periodic acid-Schiff	Slight to mod. positive	Negative
Schultz method for cholesterol and cholesterol esters	Negative	Negative
Sudan III for fat	Negative	Negative
Toluidine blue	Light blue (not metachromatic)	Light blue (not metachromatic)
von Kossa's for calcium	Negative	Negative

DISCUSSION

Reticuloendothelial hyperplasias may be divided into three types: a) inflammatory, e.g. tuberculosis or brucellosis, b) metabolic, e.g. Gaucher's disease or Niemann-Pick's disease, and c) neoplastic, e.g. leukemic or aleukemic.

The marked proliferations of histiocytes seen in lungs of the 5 mice were regarded as inflammatory responses, rather than as neoplastic or metabolic in origin, because they were accompanied by a moderate to severe chronic bronchitis, bronchiolitis and pneumonitis. Post mortem examinations revealed that the histiocytic hyperplasias were limited to the lungs. The agent responsible for the inflammatory condition of the lungs was not determined but the cellular response because mainly histiocytic, plasmacytic and lymphocytic, suggested a viral aetiology.

The cytoplasmic striations and acidophilia of the histiocytic infiltrates were strikingly consistent findings. It is not unusual to observe inclusions in histiocytes, and their staining properties depend partly upon the substance ingested. The acidophilia of the cytoplasm was probably due to phagocytosed materials. The exact nature of the fine needle-shaped streaks in the histiocytes was undetermined. Cytoplasmic staining reactions indicate that the cells contain protein material which was similar to the crystals, and similar to cytoplasmic components of the abnormal bronchial,

bronchiolar and gall bladder epithelia.

The staining reactions of histiocytes, abnormal epithelial cells and crystals with amidoblack, toluidine blue, methyl violet, congo red, pyronin, eosin, phloxine and methylene blue were the same. These staining results also indicate that the main abnormal constituent is protein and RNA.

It is postulated that the source of such protein is abnormal epithelium of bronchus, bronchioles and gall bladder. The abnormal epithelial cells were large and devoid of normal cytoplasmic content as evidenced by negative reactions to PAS, alcian blue and mucicarmine stain methods. It would seem that such proteins as well as RNA were formed or secreted by abnormal epithelial cells. These may have spilled off and crystallized, subsequently to be phagocytosed. It is the utmost interest to note that various shaped large crystals were observed in the cultivation of embryo chick tissues such as lung, cerebrum and pancreas. ¹⁷⁵ These crystals were shown to give positive response to RNA and protein staining. ¹⁷⁵ Rose, therefore, suggested that such crystals were protein products of cultivated cells and that these cells were carrying out a differentiated function of secretion.

The crystals were soft enough to cut readily in making paraffin sections and were insoluble in organic solvents such as alcohol, ether, acetone and xylene. They were, however, readily soluble in acid solutions at pH range 1-2

and in alkaline solutions at pH range 11-12.¹⁶⁴ Histochemical studies indicate that the crystals are of a protein nature.

While the crystals might be associated with a condition of sensitization or allergy, their properties are not identical with those of the Charcot-Leyden crystals as described by Wrede et al.¹⁷⁹ It has been speculated that they may be crystalline haemoglobin. The positive staining reaction with amidoblack would favour haemoglobin. However, negative reaction by benzidine indicates a lack of peroxidase which is a constituent of haemoglobin.^{163,172} A positive response of about two-thirds of the crystals to amidoblack stain is probably due to a content of protein. Amidoblack¹⁷³ is bound with proteins in staining reactions, and therefore, a positive reaction cannot be considered specific for haemoglobin. The possibility of haematoidin and bilirubin crystals can be excluded by negative response to Gmelin's^{159,172} and methylene blue staining methods.

Whether or not the presence of crystals or reticulendothelial cell hyperplasias are directly related to the acetone or 20-methylcholanthrene applications is not clear.

SUMMARY AND CONCLUSIONS

Hyperplasia of reticuloendothelial cells, presumably histiocytes, were observed in lungs and pulmonary hilar lymph nodes of five I.C.R. Swiss mice, (3 methylnholanthrene-treated and 2 acetone-treated). The histiocytes had peculiarly streaked acidophilic cytoplasm and some contained crystalloid material. Extracellular crystalloid material was also present in lungs, gall bladder and lymph nodes in two of the mice.

Hyperplasia of histiocytes in the lungs was accompanied by marked degrees of chronic bronchitis, bronchiolitis and pneumonitis, and was therefore regarded as an inflammatory rather than neoplastic form of reticuloendotheliosis.

Some of the bronchiolar, bronchial and gall bladder epithelia were morphologically and histochemically abnormal. The histochemical studies indicated that large cuboidal cells were devoid of normal cytoplasmic components and contained "abnormal" protein.

Extensive histochemical studies further indicated that both crystalloid materials and histiocytes contain protein and RNA. The abnormal appearing bronchial, bronchiolar and gall bladder mucosa are regarded as the source of the protein which is "spilled off", crystallized and then phagocytized by histiocytes.

Figures 38, 39 and 40.

Figure 38. The gross appearance of the left lung with histiocytosis, bronchitis and pneumonitis.

Compare the normal right superior lobe with the left lung which shows firm, yellowish-grey nodularities. The heart is artificially shifted to the right, and lies partly under the right superior lobe.

Figure 39. The aggregation of histiocytes in pulmonary air sacs.

There is complete replacement of alveolar spaces by many acidophilic histiocytes. Arrows point to lymphocytic infiltrations in and adjacent to a bronchus. Haematoxylin, phloxine and saffron stain. X65.

Figure 40. High magnification of histiocytes.

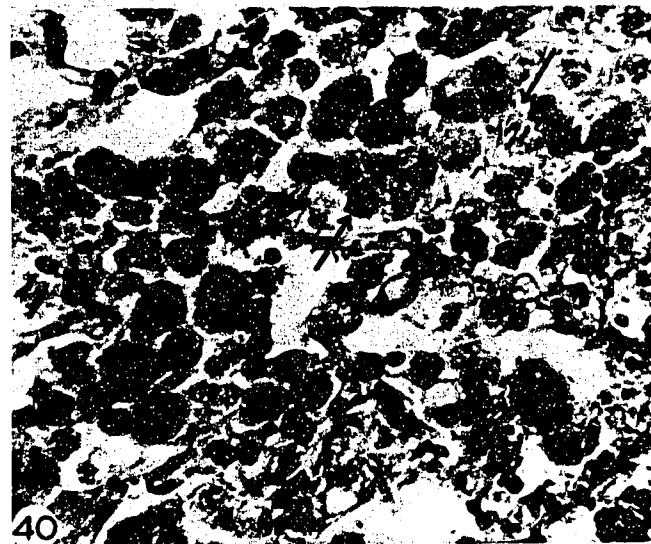
The needle-shaped striations in the cytoplasm of histiocytes are well demonstrated by Mallory's phosphotungstic acid-haematoxylin stain. X620.



38



39



40

Figures 41, 42, 43 and 44.

Figure 41. Crystals in the gall bladder.

Abnormal epithelial cells of the gall bladder are larger and markedly more acidophilic than normal epithelial cells which are seen at the lower left corner of the field. Arrows point to the normal epithelial cells. The liver parenchyma is shown on the right. Haematoxylin, phloxine and saffron stain. X225.

Figure 42. Outline drawings of crystals.

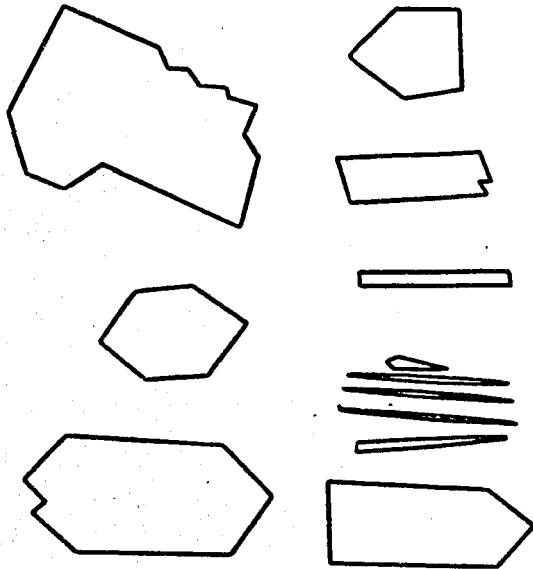
The extracellular crystals were of various shapes.

Figure 43. Photomicrograph of crystals and histiocytes.

Both crystals and histiocytes are deeply stained with phloxine. Haematoxylin, phloxine and saffron stain. X225.

Figure 44. Crystals and histiocytes stained by methyl violet.

Arrows indicate histiocytes. X225.



Figures 45, 46, 47 and 48.

Figure 45. Iron reaction of crystals and histiocytes in the lung.

A positive reaction in histiocytes is shown at the upper left corner while a negative reaction in crystals is indicated in the lower right corner of the field. Gomori's iron reaction. X225.

Figure 46. Amidoblack stain of bronchial epithelium and histiocytes.

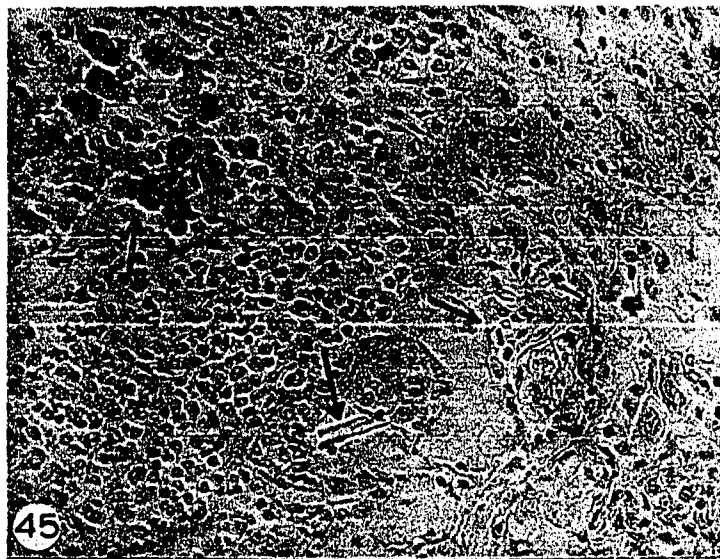
Abnormally acidophilic bronchial epithelium is positively stained. Arrows and circles indicate unstained striated histiocytes. X360.

Figure 47. Amidoblack stain of bronchial epithelium and crystals.

The rectangle encloses crystals, some epithelial cells and red blood cells that are positively stained. X180.

Figure 48. Methyl-green pyronin staining of crystals.

Crystals are stained with pyronin, indicating their RNA content. X180.



45



46



47



48

General Comment

- A. Induced Tumours.
- B. Pathogenesis of Precancerous and Cancerous Lesions.
- C. Chemical Compounds As Possible Antigens Involved
in the Production of Amyloidosis and Necrotizing
Angiitis.

A. Induced Tumours

The response to carcinogenic agents depends on the substance used, the dose administered, the solvent, the duration and the method of application and the duration of latent period. It also depends on the age and sex and strains of the animals treated.

In 1944 Cowdry and Suntzoff¹⁹¹ observed a significant increase in tumour incidence and decrease in latent period in young methylcholanthrene-treated mice as compared to old methylcholanthrene-treated mice. Bielschowsky¹⁸⁴ in 1947 showed that older female rats had a lower incidence of acetylaminofluorene induced mammary cancer than young rats. Strong²²⁶ has shown that animals from early litters develop subcutaneous sarcomas following methylcholanthrene injection far more slowly than animals from late litters of the same parents.

With small dosages of carcinogen, the response of various strains of susceptible animals tends to differ both in the incidence of induced neoplasms and in the rapidity of tumour development. With increasing dosages of carcinogen, variations in the incidence of induced neoplasm and in the rapidity of their appearance tends to diminish as the tumour incidence increases and latent period decreases.

Carcinogen induced tumours may be grouped into those induced at the site of application and those induced in a tissue or organ away from the site of application.

Among tumours induced locally, those produced by coal tar painting of the skin are best known. Following repeated applications of tar or pure carcinogenic hydrocarbons, benign papillomata develop; some of these ultimately become malignant by invading deeper tissues.²³² When carcinogenic hydrocarbons are injected subcutaneously, sarcomata may develop at the site of injection.²²⁶ The local type of carcinogenic action is well exemplified by irradiation with ultra-violet or X-ray and by the administration of the chemical carcinogens. In the present study, when uterine cervixes of mice were treated with minimal doses of a carcinogen, twelve carcinomas and two sarcomas involving the uterine cervix and vagina were encountered.

Carcinogens may induce tumours at remote sites in certain specific organs or tissues, irrespective of the manner or route of administration. An example of a tumour so induced is the interstitial cell carcinomas of the testis produced by prolonged subcutaneous injections of natural or synthetic oestrogens.^{185,197} In the present experiments, when the uterine cervixes of mice were treated with 20-methylcholanthrene, tumours developed at remote sites such as lungs, mammary glands and thymus.

Carcinogens that induce tumours at the site of application may also produce tumours in distant organs if

they are absorbed in sufficient quantity to reach and act on distant organs. However, carcinogens that induce tumours in certain specific organs and tissue remote from the site of application may not produce neoplasms at the site of application. Acetylaminofluorene that induced tumours at various sites including the auditory duct by oral administration did not produce tumours at the auditory duct when it was directly applied to the external auditory canal.²³¹ Similarly, oral administration of B-Naphtylamine induced urinary bladder tumours while in situ application of this carcinogen to the urinary bladder¹⁸⁶ failed to induce tumours in this organ.

B. Pathogenesis of Precancerous and Cancerous Lesions

After the application of 20-methylcholanthrene to the uterine cervix, invasive carcinoma was first observed 155 days after the initial application. During this "latent" period, there were changes such as acute inflammation, vacuolization, necrosis of epithelial cells, pseudoepitheliomatous hyperplasia, basal cell hyperplasia and dysplasia (atypical cell hyperplasia).

All indirect evidence supports the concept that dysplastic cells may be "precancerous" cells in the sense they may persist and eventually be transformed to "cancerous" cells. Some dysplastic cells, however, may disappear; the morphologic changes in these cells were reversible. Whether

or not the disappearance of morphologic signs of dysplasia implies the reversal of carcinogenesis is not known.

The mechanism of induced changes in dysplastic cells and cancer cells by carcinogenic hydrocarbons is not clear, but the carcinogen, in order to act, probably must penetrate cell membranes. It is not known whether or not the striking dependence of carcinogenicity on shape and size of molecules is a function of the cell membrane, which might admit molecules of a certain shape and size and bar others. Bergmann¹⁸³ postulated an acceptor for carcinogens within the cell to which only molecules of a requisite size and shape are adsorbed.

In 1947 Miller and Miller²¹⁰ demonstrated that the hepatocarcinogenic amino azo dyes are firmly bound to the protein of the liver. Since that time they have extended these findings and have obtained an impressive correlation between the process of protein binding and carcinogenesis.²¹² In 1951 Miller²¹¹ reported that the hydrocarbon, 3:4-benzopyrene, was bound to the epidermal proteins following the application to the skin of mice.

A correlation may exist between protein binding of carcinogen in breasts and mammary tumour induction. Radioactive methylcholanthrene was administered in mice by gastric intubation and the uptake by the breasts of the radioactive chemical was positively correlated both to lactation and to

tumour formation. The uptake of radioactive methylcholanthrene in breasts undergoing neoplasia was 5 - 6 times that of non-neoplastic breasts.²⁰⁵

In an endeavour to obtain information as to how the carcinogenic hydrocarbons may effect the production of tumours, studies were undertaken to observe the distribution and persistence of these agents in tissue. Studies on the distribution of the hydrocarbons were facilitated by the use of fluorescent technique. Using these methods, Beck and Peacock¹⁸² reported that the carcinogen, 3,4-benzpyrene, disappeared from the skin in 4 days after one application. Simpson and Cramer^{222,223} found that the painting of mouse skin with a benzene solution of methylcholanthrene resulted in a selective distribution of the agent. Immediately after application, the bulk of the carcinogen was observed in the epidermis at two specific sites, namely in the sebaceous glands and in the keratin layer. After 10 days, fluorescence due to the carcinogen disappeared from all parts of the skin. After intraperitoneal injection of 3,4-benzpyrene into strain A mice, the unchanged carcinogen was found in the mitochondrial and nuclear fractions of the liver of these animals.¹⁸⁶

A consideration of the relationship between the irritant and injurious effect of the carcinogen and its mode of action will now be given.

There is little doubt regarding the initial damaging effect of the carcinogen on the tissue to which it is applied. In the present experimental work, cellular damage after initial application of 20-methylcholanthrene to the uterine cervix were evidenced by vacuolization, vesiculation and necrosis of epithelial cells. In the histogenesis of carcinogen-induced tumours, there are insults to the tissue followed by hyperplastic repair and then neoplasia.¹⁹² There are examples of carcinogenesis following specific injury - for example, skin cancer after x-ray burns or prolonged exposure to ultraviolet rays,²⁰² leukemia in mice following damage to the lymphoid cells by x-ray, and carcinoma in a cirrhotic liver previously damaged by administration of carbon tetrachloride.¹⁹⁵ It has been suggested that the carcinogen itself is not a growth promoting agent, but that the latter is formed in the tissue after a specific type of damage.¹⁹³

The histological studies of Orr²¹⁴ and Pullinger²¹⁷ confirmed the postulation that carcinogens injure cells. The observations with U.V. fluorescent techniques on the response of cells to a carcinogen by Simpson and Cramer²²³ also illustrated the severe toxic effect, leading to cell degeneration, which is exerted by the carcinogen. They showed that after the initial degenerative effect of the carcinogen, a new race of cells seems to appear impervious to further damage by the carcinogen. They observed that the yellow-green fluorescent

carcinogen (e.g. methylcholanthrene), initially dissolved in the fats of the skin, invaded the cytoplasm of epithelial cells where its fluorescence changed to blue, indicating a chemical transformation. Unchanged hydrocarbon appeared in intracellular droplets in cells showing signs of degeneration. On a second application, the unchanged carcinogen penetrated completely into the cytoplasm of epithelial cells and seemed to become adsorbed on cytoplasmic granules. Hence, they concluded that the blue fluorescent metabolite conditions some cells in such a way that they are then able to take up unchanged carcinogen. Cells which do take up unchanged carcinogen undergo degeneration and are destroyed. New cells formed in consequence seem to have increased resistance to the hydrocarbon and transform it completely into the blue-fluorescent derivative. In other words, the malignant transformation may have taken place in the course of successful adaptation to the unfavourable environment presented by the carcinogen.

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Haddow proposed that when radiation was applied to a tissue a gradient of intensity lead to the death of those cells nearest the source, injury to cells further away, and no effect at all on cells at a greater distance. Among injured but surviving cells a few would be transformed into malignant cells. A similar gradient of effect was described by Peacock and Beck,²¹⁵ who found that tumour formation

occurred at a point some distance away from the sites of application of carcinogens. In other words, the tumour grows not at the place where the concentration of carcinogen would kill the cells but where survival of injured cells is just possible.

Thermal injuries are held to be responsible for tumour induction, but some features of this association are not clear. A number of authenticated cases have been described in which a skin carcinoma resulted from a local burn or scalding, either arising within a year of the injury before it had properly healed, or, in the "latent" form developing in a scar from a burn long after the initial injury.

Another important effect of a chemical carcinogen to the cells is the inhibiting influence on growth.

²⁰¹
Haddow discovered the important fact that carcinogens can act as growth inhibitors. He found that carcinogenic hydrocarbons inhibit the growth of young animals as a whole and that this effect is prolonged and specific for carcinogens and, with few exceptions, is not exhibited by related non-carcinogens nor by poisons in general. He suggested that, in tumour induction, the carcinogen acts primarily as a growth inhibitor, presenting the cell with an unfavourable environment. An adaptive and irreversible change occurs within the cell, of a qualitative or quantitative nature, leading to a new cell race with increased growth rate, lack of differentiation and increased resistance to the noxious

influence. These changes are analogous to adaptive variation of bacteria.

Three concepts are involved in the theory of cell adaptations in carcinogenesis.²²⁴

- (a) That the cancer process is, in part at least, a special type of cellular adaptation to various unusual environments, although not all cell adaptations end in cancer.
- (b) That the transformation of a normal cell to a cancer cell involves a "step-like" process. There is no clinical or experimental evidence to support the assumption that the genesis of a cancer cell involves a sudden change in a normal cell.
- (c) That the cancer process is basically a survival mechanism.

Studies of Spencer²²⁴ have suggested that there are several principles involved in the mechanism by which species become adapted to unfavourable environment.

- (a) The continuous exposure of actively multiplying free-living, bacterial species to an unfavourable environment may not be fatal to individual organism or cultures for a number of generations or cell-division cycles, but in due time the species will die.
- (b) By discontinuous or alternating exposure, an actively multiplying species can adapt and continue to survive in an environment that is fatal when the exposure is continuous.
- (c) The ability of an organism to resist an unfavourable environment is a function of its age or maturity.

(d) Organisms can resist higher intensities of an unfavourable environment during the resting stage than during the actively multiplying stage.

Normal somatic cells also have the capacity of parthenogenesis, as in germ cells. This is commonly seen in the phenomena of hyperplasia, repair, and regeneration. These processes, however, are normal reactions to injury or need. Through the ages, with increasing specialization of cells, the body has developed and maintained these homeostatic mechanisms; normal cells proliferate as needed and the process stops when they have fulfilled this biological call. Tissue regeneration and growth of the individual cells stop when an equilibrium between cells is reached. In tumour no such equilibrium is attained.

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The wide range and complexity of neoplasms is admirably explained by this concept of "somatic cell pregnancy." Known evocators of tumours have little in common, chemically or otherwise, and it has been impossible among carcinogens to find common denominators. All carcinogenic agents, however, such as solar radiation, hydrocarbons, arsenic, heat, trauma, viruses, parasites, and many others can be considered as injurious agents. The basic nature of cancer seems to be a cellular alteration which appears to be the result of cell injury by a great variety of agents. The body responds to the adverse conditions, not with the usual humoral mechanism or system of mobile defense cells, but by a fundamental

change in the exposed cells. ²²⁵

C. Chemical Compounds As Possible Antigens That Are Involved in the Production of Amyloidosis and Necrotizing Angiitis.

It is known that the carcinogens behave as haptenes or antigens in tissues. Green ¹⁹⁸ found that homologous haem-agglutinins occurred frequently in the serum of rabbits painted with active carcinogens. It is also known that polycyclic carcinogens can induce a state of hypersensitivity in the human skin. ²³⁰

According to a theory which has been put forward in various forms, an immune reaction is one component of the carcinogenic process. ^{198,199,200} A chemical carcinogen may form a complex with a specific cell protein. This complex may be antigenic, and the antibody formed to it acts on both the altered and unaltered protein, creating a state of stress in the treated tissue.

The delayed-type of hypersensitivity can be induced in adult guinea-pigs by the intradermal injection of certain chemical compounds. Landsteiner ²⁰⁷ found that picryl chloride readily united with serum protein and suggested that sensitivity is brought about by a linkage of the chemical compound with proteins of the body. Subsequent work by Tabachnick, Eisen, and Levine ²²⁷ has confirmed this view.

The occurrence of amyloidosis in various viscera and vasculitis at both the site of application and in remote

organs indicates that the chemical components themselves are responsible for these processes since saline-treated control mice did not have similar lesions. It is postulated that 20-methylcholanthrene or acetone or both of them might act as an antigen. The genesis of amyloidosis and vasculitis in methylcholanthrene or acetone-treated mice may be explained on the basis of antigen-antibody immunological mechanism.

Amyloidosis

Amyloidosis has been produced experimentally in animals such as mice, rabbits, hens, horses and ducks by the administration of casein bacteria and bacterial toxins ribonucleate, methylcholanthrene and nitrogen mustard.

203,206 181,194,196 209,213
216 221 187,190,206,228
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Rigdon applied methylcholanthrene solution to the trachea of 22 adult Pekin ducks. He observed amyloid in the liver, spleen and adrenals of 12 treated ducks. The frequency of amyloid and amount was not related to the dose of methylcholanthrene.

Evidence appears to link amyloid formation with antigen-antibody reactions. In mice, an elevation of the plasma globulins, associated with visceral deposition of amyloid, has been found to follow multiple injections of casein and the appearance of specific antibodies to this protein.

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Necrotizing Vasculitis

Vascular lesions characterized by inflammation and "fibrinoid necrosis" present a very perplexing and controversial problem. Confusion has increased because of the tendency to place the lesions in the category of periarteritis nodosa. Zeek^{233,234} recommended the noncommittal term "necrotizing angiitis" to designate the whole group generically and classified them as follows: 1) Hypersensitivity angiitis; 2) Allergic granulomatous angiitis; 3) Rheumatic arteritis; 4) Periarteritis nodosa; and 5) Temporal arteritis.

Necrotizing angiitis observed in the present study fits the criteria of a hypersensitivity angiitis.²³⁴ Hypersensitive angiitis is associated with hypersensitivity to foreign serum, sulfonamides, etc.²³⁴

There are similarities between periarteritis nodosa and hypersensitive (allergic) vasculitis, but several differences exist. In classic periarteritis nodosa, the lesions are predominantly in the medium-sized arteries, usually at the bifurcation or in the hilar regions of viscera where smaller vessels originate. Occasionally the lesions may involve small arterioles.²⁰⁸ In systemic hypersensitive angiitis, only the smallest branches of arterial and venous blood vessels are involved. All organs, including the lungs and spleen are affected and there is no predilection for the lesions to occur at the bifurcation of arterioles.²⁰⁸

Clark and Kaplan¹⁸⁹ in 1937 were the first to demon-
strate vasculitis in human serum sickness. Later, Rich²¹⁸
noted generalized, acute necrotizing vasculitis at autopsy in
patients who had received therapeutic serum, sulfonamides, or
both.

Rich and Gregory²¹⁹ noted similar lesions in 9 cases
of iodine sensitivity and produced vasculitis in animals by
inducing drug hypersensitivity. The sulfonamides, penicillin,
iodine and dilantin are the drugs most commonly responsible for
the induction of necrotizing vasculitis in human beings. Many
other compounds may, on occasion, be responsible for allergic
reactions of this type. In nearly all cases of vasculitis
induced by drugs or serum, only the small blood vessels are
involved; and in many instances, the disease is self-limited.
The lesions produced by injecting animals with horse serum,
sulfonamides, etc.,^{218,219} fit the category of systemic hyper-
sensitive angitis.

Summary and Conclusions

The application of minimal amounts of 20-methylcholanthrene to the uterine cervix of I.C.R. Swiss and phenotypically normal Pituitary Dwarf strain mice induced invasive carcinomas in 12 mice and sarcomas in 2 mice at the region of application. Some animals were allowed full life spans.

Tumours also developed at sites remote from the area of application: pulmonary tumours in 19 mice, mammary tumours in 7 mice, leukemia in 1 mouse, thymoma in 1 mouse, and jejunal adenocarcinoma in 1 mouse.

The application of 20-methylcholanthrene to the uterine cervix in mice caused acute inflammation, degeneration of epithelial cells characterized by vacuolization and vesiculation of cytoplasm, basal cell hyperplasia, pseudoepitheliomatous hyperplasia, dysplasia and finally invasive carcinoma. Metastases from 5 of the 12 carcinomas were observed.

The application of 20-methylcholanthrene to the uterine cervix of mice induced dysplasia in the cervix uteri and/or vagina in 28 mice. The concomitant dysplastic lesions were found in 2 of 12 mice bearing invasive carcinoma.

Dysplasia was preceded by invasive carcinoma but carcinoma in situ comparable to that seen in the human cervix was not noted. Dysplastic lesion in the cervix or vagina first appeared 24 days after the initial carcinogen application whereas invasive carcinoma was first observed 155 days after.

There is a minimal dose of methylcholanthrene required to produce dysplasia of the cervix and vagina in mice. Dysplasia may progress to carcinoma of the cervix without further application of the carcinogen. Unfortunately, the question as to the reversibility or irreversibility of dysplastic lesions is unanswered. However, while dysplastic changes may conceivably disappear, dysplasia was observed to persist for as long as 368 days after the discontinuation of the carcinogen treatments.

Sprague Dawley rats given 20-methylcholanthrene (1.8 ml. or 2.2 ml.) had no significant local changes during 390 days of observation. (This negative response may be attributable to insufficient dosage or strain resistance; possible, however, a prolonged latent period has not yet terminated. Observations upon these animals will continue to full life spans).

A total of 102 pulmonary tumours in 19 mice were classified histologically. 98 were benign (adenoma) and 4 were malignant (adenocarcinoma). The tumour cells were regarded as derived from alveolar lining cells.

Thirteen mammary tumours in 7 methylcholanthrene-treated mice and 1 acetone-treated mouse were malignant histologically and 2 of the mice had lung metastases. Three of 13 tumours had squamous metaplasia (adenocanthoma).

Necrotizing angiitis in the uterine cervix, vagina, lungs, kidneys, ovaries and other sites developed in 7 methylcholanthrene-treated and 6 acetone-treated mice.

Amyloidosis was found in 4 methylcholanthrene-treated and 2 acetone-treated mice in various organs such as liver, spleen, kidneys, lymph nodes, heart and adrenal glands.

Carcinogenic or non-carcinogenic chemical compounds may act as a haptene or may themselves possess antigenic action. The amyloidosis and necrotizing angiitis were considered to be antigen-antibody reactions.

Hyperplasias of histiocytes were observed in lungs and pulmonary hilar lymph nodes of three methylcholanthrene-treated and two-acetone-treated I.C.R. Swiss mice. Some of histiocytes contained crystalloid materials. Extracellular crystals were also present in lungs, gall bladder and lymph nodes in two of these mice. The histiocytic hyperplasias were regarded as inflammatory responses. The crystalloid material contained protein and RNA.

Chemical carcinogens injure cells and the cells respond by degeneration, necrosis or hyperplasia. In the course of carcinogenesis, the presence of "precancerous" cells which eventually become neoplastic is postulated. Postulates regarding "growth inhibition", "adaptation" of cells or somatic cell "parthenogenesis" may explain some aspects of carcinogenesis.

Bibliography

Induced Dysplasia and Carcinoma of the Uterine Cervix and Vagina
in Mice and Rats.

1. Ayre, J.L. Role of the halo cell in cervical cancer-igenesis. A virus manifestation in premalignancy? *Obst. & Gynec.* 15: 481-491, 1960.
2. Bernhard, W. Electron microscopy of tumor cells and tumor viruses; a review. *Cancer Research* 18: 491-509, 1958.
3. Broghamer, W.L., Jr., and Christopherson, W.H. An interferometric study of the anhydrous nuclear mass of exfoliated cells from experimental cervical cancer. *Cancer* 14: 378-383, 1961.
4. Carson, R.P., and Gall, E.A. Preinvasive carcinoma and precancerous metaplasia of the cervix. *Am. J. Path.* 30: 15-29, 1954.
5. Cherry, C.P., and Glucksmann, A. The effect of endocrine changes of irradiation and of additional treatment of the skin on the induction of tumours in the female genital tract of rats by chemical carcinogens. *Brit. J. Cancer* 14: 489-501, 1960.
6. Christopherson, W.H., and Broghamer, W., Jr. Progression of experimental cervical dysplasia in the mouse. *Cancer* 14: 201-204, 1961.
7. Cioli, R. Tumori sperimentali da catrame dell'utero e loro sviluppo in rapporto alla funzione ovarica. *Boll. e atti d.r. Acad. med. di Roma* 55: 291-296, 1929.

8. Dobrobołoskaia-Zavadskaia, H., and Simsilevich, F.
Au sujet de la réaction des organes genitaux de la souris aux instillations du 1:2:5:6 dibenzanthracene dans le vagin. Libro de oro, pp. 293-301, 1935.
9. Fidler, H.K., and Boyes, D.A. Probable percentage of invasive carcinoma passing through the stage of carcinoma in situ. Acta Cytol. 6:118, 1962.
10. Fishman, H., Shear, H.J., Friedman, H.F., and Stewart, H.L. Studies in carcinogenesis. XVII. Local effects of repeated application of 3,4-benzpyrene and of human smegma to the vagina and cervix of mice. J.Nat. Cancer Inst. 2:361-367, 1941-1942.
11. Galvin, G.A., Jones, H.W., and TeLinde, R.W. The significance of basal cell hyperactivity in cervical biopsies. Am. J. Obst. & Gynec. 70: 808-821, 1955.
12. Gardner, W.U. Uterine cervical and vaginal cancers in experimental animals. Acta Un. int. Cancr. 17: 905-909, 1961.
13. Gardner, W.U., and Pan, S.C. Malignant tumors of the uterus and vagina in untreated mice of the PM stock. Cancer Research 8: 241-256, 1948.
14. Gardner, W.U., Allen, E., Smith, G.H., and Strong, L.C. Carcinoma of the cervix of mice receiving estrogens. J.A.M.A. 110: 1182-1183, 1938.

15. Gardner, W.U., and Allen, E. Malignant and non-malignant uterine and vaginal lesions in mice receiving estrogens and estrogens and androgens simultaneously. *Yale J. Biol. & Med.* 12: 213-234, 1939.
16. Gardner, W.U. Carcinoma of uterine cervix and upper vagina: Induction under experimental conditions in mice. *Ann. New York Acad. Sc.* 75: 543-564, 1959.
17. Hanau, A.H. *Fortschr. Med.* 7: 321, 1889. Quoted by Peacock, P.R., in *Cancer Vol. 1*, 1957 pp 32-75, Butterworth & Co. (London).
18. Heins, H.C. Jr., Dennis, E.J., and Pratt-Thomas, H.R. Possible role of smegma in carcinoma of the cervix. *Am. J. Obst. & Gynec.* 76: 726-735, 1958.
19. Kaminetzky, H.A. Human cervical epithelial changes produced by podophyllin. *Am. J. Obst. & Gynec.* 80: 1055-1062, 1960.
20. Kaminetzky, H.A., McGrew, E.A., and Phillips, R.L. Experimental cervical epithelial dysplasia. *Obst. & Gynec.* 14: 1-10, 1959.
21. Kaminetzky, H.A., and McGrew, E.A. Podophyllin and mouse cervix, effect of long-term application. *Arch. Path.* 73: 481-485, 1962.
22. Kaminetzky, H.A., and Swerdlow, H. Atypical cervical epithelial hyperplasia. *Am. J. Obst. & Gynec.* 82: 903-908, 1961.

23. Koprowska, I., and Bogacz, J. A cytopathologic study of tobacco tar-induced lesions of uterine cervix of mouse. *J. Nat. Cancer Inst.* 23: 1-19, 1959.
24. Koss, L.G. Diagnostic cytology and its histopathologic bases. 1961, pp 112-114, J.B. Lippincott Co.
25. Koss, L.G. Diagnostic cytology and its histopathologic bases. 1961, pp 128-129, J.B. Lippincott Co.
26. Lever, W.F. Histopathology of the skin. 1949, pp 288-290, J.B. Lippincott Co.
27. Lever, W.F. Histopathology of the skin. 1949, pp 291-294, J. B. Lippincott Co.
28. Loeb, L., Burns, E.L., Suntzeff, V., and Moskop, H. Carcinoma-like proliferations in vagina, cervix and uterus of mouse treated with estrogenic hormones. *Proc. Soc. Exper. Biol. & Med.* 35: 320-322, 1936.
29. McGrew, E.A., and Kamnietzky, H.. The genesis of experimental cervical epithelial dysplasia. *Am. J. Clin. Path.* 35: 538-548, 1961.
30. McKay, D.G. et al. Clinical and pathologic significance of anaplasia (atypical hyperplasia) of the cervix uteri. *Obst. & Gynec.* 13: 2-21, 1959.
31. Montgomery, H., and Waisman, H. Epithelioma attributable to arsenic. *J. Invest. Dermatol.* 4: 365-383, 1941.
32. Murphy, E.D. Studies on carcinogen-induced carcinoma of the cervix of mice. *Am. J. Path* 29: 608, 1953.

33. Novak, E.R., and Galvin, G.A. Mistakes in interpretations of intra-epithelial carcinoma. *Am. J. Obst. & Gynec.* 62: 1079-1085, 1951.
34. Novak, E., and Novak, E.R. *Gynecologic and Obstetric Pathology*, 4th. ed. 1958, pp 102.
35. Overholser, H.D., and Allen, E. Ovarian hormone and traumatic stimulation of monkey's cervix to a condition resembling early cancer. *Proc. Soc. Exper. Biol. & Med.* 30: 1322-1326, 1933.
36. Pan, S.C., and Gardner, W.U. Carcinoma of the uterine cervix and vagina in estrogen and androgen-treated hybrid mice. *Cancer Research* 8: 337-345, 1948.
37. Papanicolaou, G.H. A new procedure for staining vaginal smears. *Science* 95: 438-439, 1942.
38. Peckham, B., and Greene, R.R. Follow-up on cervical epithelial abnormalities. *Am. J. Obst. & Gynec.* 74: 804-815, 1957.
39. Perry, I.H., and Ginzton, L.L. The development of tumors in female mice treated with 1:2:5:6 - Dibenzanthracene and Thioelin. *Am. J. Cancer* 29: 680-704, 1937.
40. Pfeiffer, C.A., and Allen, E. Attempts to produce cancer in Rhesus monkeys with carcinogenic hydrocarbons and estrogens. *Cancer Research* 8: 97-127, 1948.
41. Pott, P. *Chirurgical observations, etc.*, London 61-68, 1775, quoted by Peacock, P.R., in *Cancer Vol. 1*, 1957 pp 32-75, Butterworth & Co. (London).

42. Pratt-Thomas, H.R., Heins, H.C., Latham, E., Dennis, E.J., and McIver, F.A. The carcinogenic effect of human smegma: An experimental study. I. Preliminary report. *Cancer* 9: 671-680, 1956.
43. Reagan, J.W., Seidemann, I.L., and Saracusa, Y. The cellular morphology of carcinoma-in-situ and dysplasia or atypical hyperplasia of the uterine cervix. *Cancer* 6: 224-235, 1953.
44. Reagan, J.W., Hicks, D.J., and Scott, R.B. Atypical hyperplasia of the uterine cervix. *Cancer* 8: 42-52, 1955.
45. Reagan, J.W., Wentz, W.B., and Machicao, H. Induced cancer of the cervix uteri in the mouse. *Arch. Path.* 60: 451-461, 1955.
46. Reagan, J.W., and Wentz, W.B. Changes in mouse cervix antedating induced cancer. *Cancer* 12: 389-395, 1959.
47. Sađirođlu, H. The nature of the perinuclear halo: Further clinical cytological and pathological studies. *Am. J. Obst. & Gynec.* 77: 159-174, 1959.
48. Sađirođlu, H. Progression and regression studies of precancer (anaplastic or dysplastic) cells, and the halo test. *Am. J. Obst. & Gynec.* 85: 454-469, 1963.
49. Saphir, O., Leventhal, H.L., and Kline, T.S. Podophyllin-induced dysplasia of the cervix uteri. *Am. J. Clin. Path.* 32: 446-456, 1959.

50. Scapier, J., Day, E., and Durfee, G.H. Intra-epithelial carcinoma of cervix, cytohistological and clinical study. *Cancer* 5: 315-323, 1952.
51. Slye, H., Holmes, H.F., and Wells, H.G. Primary spontaneous tumors of the uterus in mice with a review of comparative pathology of uterine neoplasm; studies on the incidence and the heritability of spontaneous tumors in mice. *J. Cancer Research* 8: 96-118, 1924.
52. Stewart, F.H. Factors influencing curability of cancer. In proceedings of the Third National Cancer Conference. 1957, pp 62-73, J.B. Lippincott Co.
53. Suntzeff, V., Burns, E.L., Moskop, H., and Loeb, L. On the proliferative changes taking place in the epithelium of the vagina and cervix of mice with advancing age and under the influence of experimentally administered estrogenic hormones. *Am. J. Cancer* 32: 256-289, 1938.
54. Suntzeff, V., and Carruthers, C. Water content in epidermis of mice undergoing carcinogenesis by methylcholanthrene. *Cancer Research* 6: 574-577, 1946.
55. Thiery, H. et al. Virus-like particles in chemically induced carcinoma of the uterine cervix. *Nature (London)* 183: 694-695, 1959.
56. Tsutsui, H. Induced canceroid. *Gann* 12:17, 1918.
57. Vellios, F., and Griffin, J. The pathogenesis of dimethylbenzanthracene-induced carcinoma of the cervix of rats. *Cancer Research* 17: 364-366, 1957.

58. von Haan, E., and Menzies, P. Experimental studies in exfoliative cytology. Proc. Am. Assoc. Cancer Research 1: 22, 1953.
59. von Haan, E., and Scarpelli, D.G. Experimental carcinoma of the cervix: A comparative cytologic and histologic study. Cancer Research 15: 449-455, 1955.
60. von Volkmann, R. Berl. Klin. Wschr. 11: 218, 1874, quoted by Peacock, P.R., in Cancer Vol. 1, 1957, pp 32-75, Butterworth & Co. (London).
61. Weiner, I., Burke, L., and Goldberger, H.A. Carcinoma of the cervix in Jewish women. Am. J. Obst. & Gynec. 61: 418-422, 1951.
62. Williams, F.L., Paullus, K., and Erickson, C.C. Proliferative epithelial changes and carcinoma of cervix and vagina in mice with prolonged administration of alpha estradiol-sensitized collodion particles. Proc. Am. Assoc. Cancer Research 1: 60, 1953.
63. Yanagiwa, K., and Ichikawa, K. Experimental study of the pathogenesis of carcinoma. J. Cancer Research 3: 1-29, 1918.
64. Young, P.A., Hertig, A.T., and Armstrong, D. A study of 135 cases of carcinoma in situ of the cervix at the Free Hospital for Women. Am. J. Obst. & Gynec. 58: 867-895, 1949.
65. Zinser, H.K. Tissue culture and carcinoma in situ. Acta Cytol. 6: 159-160, 1962.

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of 20-Methylcholanthrene to the Uterine Cervix

66. Andervont, H.B. Pulmonary Tumors in mice: IV. Lung tumors induced by subcutaneous injection of 1,2,5,6-Dibenzanthracene in different media and by its direct contact with lung tissues. *Publ. Hlth. Rep.* 52: 1584-1589, 1937.
67. Biancifiori, C., and Ribacchi, R. Pulmonary tumours in mice induced by oral isoniazid and its metabolites. *Nature* 194: 488-489, 1962.
68. Bittner, J.J. Spontaneous incidence of lung tumors in relation to the incidence of mammary tumors in an inbred strain of albino mice (Strain A). *Am. J. Cancer* 27: 519-524, 1936.
69. Bonne, C. Morphological resemblance of pulmonary adenomatosis (Jaagsiekte) in sheep and certain cases of cancer of the lung in man. *Am. J. Cancer* 35: 491-501, 1939.
70. Campbell, J.A. The effects of road dust "freed" from tar products upon the incidence of primary lung tumours of mice. *Brit. J. Exper. Path.* 18: 215-223, 1937.
71. Cember, H., and Watson, J.A. Bronchogenic carcinoma from radioactive barium sulfate. *A.I.A. Arch. Industr. Health* 17: 230-235, 1958.
72. Daido, S. Morphological studies on experimental murine lung tumor induced by abdominal injection of urethane. I. Macroscopic and statistical study. *Kyoto Daig. Kekkaku Ken.* 9: 164-168, 1961.

73. Daido, S. II. Histological study. *Ibid* 9:169-174, 1961.
74. Daido, S. III. Electron microscopical study. *Ibid* 9: 175-185, 1961.
75. Della Porta, G., Kolb, L., and Shubick, P. Induction of tracheobronchial carcinomas in the Syrian golden hamster. *Cancer Research* 18: 592-597, 1958.
76. Furth, J., and Furth, O.B. Monocytic leukemia and other neoplastic diseases occurring in mice following intrasplenic injection of 1:2-benzpyrene. *Am. J. Cancer* 34: 169-183, 1938.
77. Gates, O., and Warren, S. Production of bronchial carcinomas in mice. *Am. J. Path.* 36: 653-671, 1960.
78. Gates, O., and Warren, S. Histogenesis of lung carcinoma in mice induced by gamma radiation. *Arch. Path.* 71: 693-713, 1961.
79. Grady, H.G., and Stewart, H.L. Histogenesis of induced pulmonary tumors in strain A mice. *Am. J. Path.* 16: 417-432, 1940.
80. Haaland, H. Fourth scientific report of the imperial cancer research fund, London, 1911, p. 1, quoted by Magnus, H.A. in *J. Path. & Bact.* 49: 21-31, 1939.
81. Heston, W.E., and Dunn, T.B. Tumor development in susceptible strain A and resistant strain L lung transplants in LAF₁ hosts. *J. Nat. Cancer Inst.* 11: 1057-1071, 1951.

82. Horn, H. A., Congdon, C.C., Eschenbrenner, A.B., Andervont, H.B., and Stewart, H.L. Pulmonary adenomatosis in mice. *J. Nat. Cancer Inst.* 12: 1297-1316, 1952.
83. Horning, E.S. Induction of bronchogenic carcinomas in mice. *Lancet* 2: 207-209, 1947.
84. Horning, E.S. Studies on the induction of lung cancer in mice. *Brit. J. Cancer* 4: 235-244, 1950.
85. Jobling, J.W. Monographs of the Rockefeller Institute for Medical Research No. 1, 1910, pp.81, quoted by Magnus, H.A. *J. Path. & Bact.* 49:21-31, 1939.
86. Klärner, P. Zur Ultrastruktur des Urethane-Lungentumors der Maus. *Naturwissenschaften* 47: 66-67, 1960.
87. Klärner, P., and Giesecking, R. Zur Ultrastruktur des Lungentumors des Maus. *Z. Krebsforsch.* 64: 7-21, 1960.
88. Kotin, P., and Falk, H.L. Experimental induction of pulmonary tumors in strain A mice after their exposure to an atmosphere of ozonoid gasoline. *Cancer* 9: 910-917, 1956.
89. Lisco, H. Autoradiographic and histopathologic studies in radiation carcinogenesis of lung. *Lab. Invest.* 8: 162-170, 1959.
90. Livingood, L.E. Tumors in the mouse. *Johns Hopkins Hosp. Bull.* 7: 177-178, 1896.
91. Lorenz, E., Heston, W.E., Deringer, M.K., and Eschenbrenner, A.B. Increase in incidence of pulmonary tumors in strain A mice following long continued irr-

- adiation with gamma-rays. *Cancer Research* 6:485-486, 1946.
92. Lorenz, E., Heston, W.E., Deringer, M.K., and Eschenbrenner, A.B. Increase in incidence of lung tumors in strain A mice following long continued irradiation with gamma-rays. *J. Nat. Cancer Inst.* 6: 349-353, 1946.
93. Lynch, C.J. Studies on the relation between tumors susceptibility and heredity. III. Spontaneous tumors of the lung in mice. *J. Exper. Med.* 43: 339-355, 1926.
94. Lynch, C.J. Studies on relation between tumor susceptibility and heredity; inheritance of susceptibility to tar-induced tumors in lungs of mice. *J. Exper. Med.* 46: 917-933, 1927.
95. Lynch, C.J. Studies on relation between tumor susceptibility and heredity; influence of heredity upon incidence of lung tumors in mice. *J. Exper. Med.* 54: 747-760, 1931.
96. Lynch, C.J. Susceptibility of mouse strains to lung tumor and sarcoma induced by 1:2:5:6-dibenzanthracene. *Proc. Soc. Exp. Biol. & Med.* 33: 401-403, 1935-1936.
97. Lynch, C.J. Cancer problem symposium. 1937, pp.22-28. *Am. Assoc. Advancement of Science.*
98. Magnus, H.A. The experimental production of malignant papillomata of the lung in mice with 1:2:5:6-dibenzanthracene. *J. Path. & Bact.* 49: 21-31, 1939.

99. Moller, P. Carcinoma pulmonaire primaire chez les rats pie badigeonnes au goudron. Acta Path. Microbiol. Scand. 1: 412-437, 1924.
100. Mori, K., Yasuno, A., and Matsumoto, K. Induction of pulmonary tumors in mice with isonicotinic acid hydrazide feeding. Gann 50: 123, (Supp.) 1959.
101. Mori, K., and Yasuno, A. Induction of pulmonary tumors in mice by subcutaneous injection of 4-nitroquinoline N-oxide. Gann 52: 149-153, 1961
102. Mori, K. Preliminary note on adenocarcinoma of the lung in mice induced with 4-nitroquinoline N-oxide. Gann 52- 265-270, 1961.
103. Mostofi, F.K., and Larsen, C.D. Histopathogenesis of pulmonary tumors induced in strain A mice by urethan. J. Nat. Cancer Inst. 11: 1187-1221, 1951.
104. Murphy, J.B., and Sturn, E. Primary lung tumors in mice following the cutaneous application of coal tar. J. Exp. Med. 42: 693-700, 1925.
105. Nettleship, A., Henshaw, P.S., and Meyer, H.L. Induction of pulmonary tumors in mice with ethyl carbamate (urethane). J. Nat. Cancer Inst. 4:309-319, 1943-1944.
106. Okada, Y., Daido, S., and Ishiko, S. Morphological studies of induced pulmonary tumors in mice with special reference to their cytogenesis. Acta Tuberculosea Jap. 11: 73-82, 1962.

107. Orr, J.W. The induction of pulmonary adenomata in mice by urethane. *Brit. J. Cancer* 1: 311-316, 1947.
108. Orr, J.W. The histology and histogenesis of pulmonary adenomata of mice. *Ibid* 1: 316-322, 1947.
109. Passey, R.D., Leese, A., and Knox, J.C. Bronchiectasis and metaplasia in the lung of the laboratory rat. *J. Path. Bact.* 42: 425-434, 1936.
110. Schabad, L.H. Quelques données expérimentales sur les tumeurs du pounon. *Acta Un. int. Cancr.* 3: 189-198, 1938.
111. Seeling, H.G., and Benignus, E.L. Coal smoke soot and tumors of lung in mice. *Am. J. Cancer* 28: 96-111 1936.
112. Seeling, H.G., and Benignus, E.L. Lung tumor development in a resistant strain of mice subjected to inhalation of soot. *Am. J. Cancer* 34: 391-398, 1938.
113. Shinkin, M.B. Production of lung tumors in mice by intratracheal administration of carcinogenic hydrocarbons. *Am. J. Cancer* 35: 538-542, 1939.
114. Shinkin, M.B. Induced pulmonary tumors in mice. I. Susceptibility of seven strains of mice to the action of intravenously injected methylcholanthrene. *Arch. Path.* 29: 229-238, 1940.
115. Shinkin, M.B., and McClelland, J.H. Induced pulmonary tumors in mice: IV. Analysis of dose response data with methylcholanthrene. *J.Nat. Cancer Inst.* 10:597-603,1949.

116. Slye, M., Holmes, H.F., and Wells, H.G. The primary spontaneous tumors of the lungs in mice; studies on the incidence and inheritability of spontaneous tumors in mice. J. Med. Research 30: 417-442, 1914.
117. Smith, W.E. The tissue transplant technic as a means of testing materials for carcinogenic action. Cancer Research 9: 712-723, 1949.
118. Smith, W.E. The neoplastic potentialities of mouse embryo tissues: V. Tumors elicited with methylcholanthrene from pulmonary epithelium. J. Exp. Med. 91: 87-104, 1950.
119. Stewart, H.L. Pulmonary tumors in mice. Pathology of Cancer, 2nd Ed. 1959, pp. 18-37.
120. Svoboda, D.J. Ultrastructure of pulmonary adenomas in mice. Cancer Research 22: 1197-1201, 1962.
121. Tyzzer, E.E. A series of twenty spontaneous tumors in mice, with the accompanying pathological changes and the results of the inoculation of certain of these tumors into normal mice. J. Med. Research 17: 155-197, 1908.
122. Tyzzer, E.E. A series of spontaneous tumors in mice with observations on the influence of heredity on the frequency of their occurrence. J. Med. Research 21: 479-518, 1909.

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20-Methylcholanthrene to the Uterine Cervix

123. Andervont, H.B., and Dunn, T.B. Response of mammary-tumor-agent free strain DBA mice to percutaneous application of methylcholanthrene. J. Nat. Cancer Inst. 10: 895-925, 1950.
124. Biancifiori, C., Bonser, G.M., and Caschera, F. Ovarian and mammary tumours in intact C₃Hb virgin mice following a limited dose of four carcinogenic chemicals. Brit. J. Cancer 15: 270-283, 1961.
125. Bittner, J.J. Some possible effects of nursing on the mammary gland tumor incidence in mice. Am. J. Clin. Path. 7: 430-435, 1937.
126. Bittner, J.J., and Little, C.C. The transmission of breast and lung cancer in mice. J. Hered. 28: 117-121, 1937.
127. Bittner, J.J. Experimental aspects of mammary carcinoma in mice, Ch. IV, Breast cancer and its diagnosis and treatment. ed. Lewison, E.F., 1955, pp. 75-102, Williams and Wilkins Co., Baltimore.
128. Bonser, G.M. The evolution of mammary cancer induced in virgin female IF mice with minimal doses of locally-acting methylcholanthrene. J. Path. & Bact. 68: 531-546, 1954.
129. Crisp. Malignant tumour on the pectoral muscle of a mouse. Tr. Path. Soc. London 5: 348, 1854, quoted by

- Dunn, T.B. in Morphology and histogenesis of mammary tumors, A symposium on Mammary Tumors in Mice, 1945 pp. 13-38, Am. Ass. for Advancement of Science, Wash. D.C.
130. Englebreth-Holm, J. Acceleration of development of mammary carcinomas in mice by methylcholanthrene. *Cancer Research* 1: 109-112, 1941.
131. Geyer, R.P. et al. Effect of dose and hormones on tumor production in rats given emulsified 9,10-dimethyl-1,2-benzanthracene intravenously. *Cancer Research* 13: 503-506, 1953.
132. Grüneberg, H. The genetics of the mouse. 2nd. ed., 1952, pp.438-449, The Hague Martinus Nijhoff.
133. Heston, W.E., and Andervont, H.B. Importance of genetic influence on occurrence of mammary tumors in virgin female mice. *J. Nat. Cancer Inst.* 4: 403-407, 1944.
134. Huggins, C., Grand, L.C., and Brillanets, F.P. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. *Nature* 189: 204-207, 1961.
135. Huggins, C., Morii, S., and Grand, L.C. Mammary cancer induced by a single dose of polynuclear hydrocarbons: Routes of administration. *Ann. Surg.* 154: 315-318, 1961 (Suppl).
136. Jensen, C.O. Experimentelle Untersuchungen über Krebs bei Mäusen. *Centbl. f. Bakt.* 34: 28 and 122, 1903.

137. Jones, E.E. Tumor incidence in Line A albino mice following injections of progynon-B. *Am. J. Cancer* 39: 94-99, 1940.
138. Khanolkar, V.R. Functional components of chemically induced mammary cancer. *Acta Un. int. Cancer.* 17: 45-54, 1961.
139. Kirschbaum, A., Williams, W.L., and Bittner, J.J. Induction of mammary cancer with methylcholanthrene. I. Histogenesis of the induced neoplasm. *Cancer Research* 6: 354-362, 1946.
140. Lathrop, A.E.C., and Loeb, L. Further investigations on the origin of tumors in mice. III. On the part played by internal secretion in the spontaneous development of tumors. *J. Cancer Research* 1: 1-20, 1916.
141. Livingood, L.E. Tumors in the mouse. *Johns Hopkins Hosp. Bul.* 7: 177-178, 1896.
142. Maisin, J., and Coolen, M-L. Au sujet du pouvoir cancérogène du méthylcholanthrène. *Compt. rend. Soc. Biol.* 123: 159-160, 1936.
143. Marchant, J. Chemical induction of breast tumours in mice of the C₅₇B1 strain. The influence of pseudo-pregnancy, pregnancy and lactation on induction by methylcholanthrene. *Brit. J. Cancer* 15:568-573, 1961.
144. Miller, E.W., and Pybus, F.C. Effect of oestrone on mice of three inbred strains, with special reference to mammary glands. *J. Path. Bact.* 54:155-168, 1942.

145. Mühlbock, O. The hormonal genesis of mammary cancer. *Advanc. Cancer Research* 4: 371-391, 1956.
146. Murray, W.S. Ovarian secretion and tumor incidence. *Science* 66: 600-601, 1927.
147. Murray, W.S., Ovarian secretion and tumor incidence. *J. Cancer Research* 12: 18-25, 1928.
148. Murray, W.S., and Little, C.C. Extrachromosomal influence in relation to the incidence of mammary and non-mammary tumor in mice. *Am. J. Cancer* 27:516-518, 1936.
149. Orr, J.W. Methylcholanthrene-induced mammary tumours in a low-breast-cancer strain of mice. *Acta. Un. int. Cancr.* 7: 294-298, 1951.
150. Scholler, J., and Carnes, R.E. Preliminary studies with mammary tumors in the rat induced with 7,12-dimethylbenz (a)anthracene (DMBA). *Proc. Am. Assoc. Cancer Research* 2: 343, 1958.
151. Shay, H., Harris, C., and Gruenstein, M. Influence of sex hormones on the incidence on a mouse mammary carcinoma. *Cancer Research* 12: 713-715, 1952.
152. Shimkin, H.B. Conclusions - including discussion of the possible implications for man. In a Symposium on Mammary Tumors in Mice. 1945, pp. 209-223, Am. Ass. for advancement of Science, Wash., D.C.

153. Strong, L.C., and Williams, W.L. Genetic analysis of induction of tumors by methylcholanthrene; local and remote induction of carcinoma of mammary gland. Cancer Research 1: 886-890, 1941.
154. Squartini, F. Strain differences in growth of mouse mammary tumors. J. Nat. Cancer Inst. 26: 813-828, 1961.
155. Wilson, R.H., DeEds, F., and Cox, A.J., Jr. The toxicity and carcinogenic activity of 2-acetaminofluorene. Cancer Research 1: 595-608, 1941.

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Material in Laboratory Mice

156. Abrikossoff, A., and Wulff, F. Ueber Eiweisskristallbildung in einem Fall von Nyclon. Verhandl. d. deutsch. path. Gesellsch 22: 270-277, 1927.
157. Agress, H., and Smith, H.G. Purpura haemorrhagica associated with widespread deposits of crystalline material: Reticuloendotheliosis. Arch. Path. 29: 553-560, 1940.
158. Brandts, C.E. Über Eisenschlüsse im Kern der Leberzelle und ihre Beziehungen zur Pigmentbildung (a) beim Hund(b) beim Menschen. Beitr. path. Anat. 45: 457-475, 1909.
159. Culling, C.F.A. Handbook of Histopathological Technique. 1957, Butterworth & Co. (London).
160. Dubreuil, G., and Favre, H. Arch. d'anat. micr. 17: 302, 1920-1921, quoted by Agress, H., and Smith, H.E. in Arch. Path. 29: 553-560, 1940.
161. Dunn, T.B. Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J. Nat. Cancer Inst. 14: 1281-1433, 1954.
162. Glaus, A. Virchows Arch. f. path. Anat. 223: 301-339, 1916-1917, quoted by Agress, H., and Smith, H.C. in Arch. Path 29: 553-560, 1940.

163. Gomori, G. Microscopic Histochemistry, Principles and Practice. 1952, The University of Chicago Press.
164. Green, E.U. On the occurrence of crystalline material in the lungs of normal and cancerous Swiss mice. Cancer Research 22: 210-217, 1942.
165. Gridley, M.F. Manual of Histologic and Special Staining Technics. 1957, Armed Forces Inst. of Path., Wash., D.C.
166. Haaland, H. Les tumeurs de la souris. Ann. Inst. Pasteur 19: 165-207, 1905.
167. Horn, H.A., Congdon, C.C., Eschenbrenner, A.B., Lindervont, H.B., and Stewart, H.L. Pulmonary adenomatosis in mice. J. Nat. Cancer Inst. 12: 1297-1316, 1952.
168. Maximow, A.A. Bindegewebe und blutbildende Gewebe, in Hüllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, Vol 2, Pt. 1, P. 262. quoted by Ritchie, G., and Meyer, O.O. in Reticulo-endotheliosis. Arch. Path. 22: 729-737, 1936.
169. Nicolau, S., and Kopciowska, L. Sur les manifestations d'un ultravirus "saprophyte" dans l'organisme du chien. Ann. Inst. Pasteur 57: 244-253, 1936.
170. Opstad, A.H. A methyl green-pyronin stain for plasma cells in tissues. Stain Tech. 34: 293, 1959.

171. Osgood, E.H., and Ashworth, C.H. Atlas of Haematology, 1937, pp. 102, fig 307, J.W. Stacey, Inc., San Francisco.
172. Pearse, A.G.E. Histochemistry, Theoretical and Applied, 1953, J. & A. Churchill, Ltd., London.
173. Puchtler, H. Amidoblack as a stain for haemoglobin. Arch. Path. 73: 245-294, 1962.
174. Ritchie, G., and Meyer, O.O. Reticulo-endotheliosis. Arch. Path. 22: 729-737, 1936.
175. Rose, G.G. Biologic crystals and particles produced in tissue culture. Cancer Research 23: 279-284, 1963.
176. Tyzzer, E.E. A series of spontaneous tumors in mice with observations on the influence of heredity on the frequency of their occurrence. J. Med. Research 21: 479-518, 1909.
177. Thompson, S.H. II, Cook, J.E., and Hoey, H. Histochemical studies of acidophilic crystalline intranuclear inclusions in the liver and kidney of dogs. Am. J. Path. 35: 607-623, 1959.
178. Weatherford, H.L. Intranuclear crystals in the hepatic cells of Canidae-wolves, foxes, jackals and non-domestic dogs. Anat. Rec. 73: 29-37, 1939.
179. Wrede, F., Boldt, F., and Buch, E. Über die Natur der Charcot-Leyden-Böttcher-Heumann Krystalle. Ztschr. f. physiol. Chem. 165: 155-166, 1927.

General Comments

180. Auerbach, O., and Stemmerman, H.G. Renal amyloidosis. Arch. Int. Med. 74: 244-253, 1944.
181. Bailey, C.H. The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli. J. Exp. Med. 23: 773-790, 1916.
182. Beck, S., and Peacock, P.R. Latent carcinogenic action of 3:4-benzpyrene; results of intermittent applications to skin of mice. Brit. J. Exper. Path. 21: 227-230, 1940.
183. Bergmann, F. On mechanism of tumor production by chemical agents. Cancer Research 2: 660-663, 1942.
184. Bielschowsky, F. Carcinogenic action of 2-acetylaminofluorene and related compounds. Brit. Med. Bull. 4: 382-385, 1947.
185. Bonser, G.H., and Robson, J.H. The effect of prolonged oestrogen administration upon male mice of various strains: development of testicular tumours in the Strong A strain. J. Path. Bact. 51: 9-22, 1940.
186. Bonser, G.H., and Jull, J.W. 30th Ann. Rept. Brit. Emp. Cancer Campaign pp. 180, 1952.
187. Caesar, R. Die Feinstruktur von Milz und Leber bei experimenteller Amyloidose. Z. Zellforsch. 52: 653-673, 1960.

188. Calcutt, G., and Payne, S. Intracellular distribution of 3:4-benzpyrene during metabolism in mouse liver. *Brit. J. Cancer* 7: 279-282, 1953.
189. Clark, E., and Kaplan, B.I. Endocardial, arterial, and other mesenchymal alterations associated with serum disease in man. *Arch. Path.* 24: 458-478, 1937.
190. Cohen, A.S., Calkins, E., and Levene, C.J. Studies on experimental amyloidosis. I. Analysis of histology and staining reactions of casein-induced amyloidosis in the rabbit. *Am. J. Path.* 35: 971-989, 1959.
191. Cowdry, E.V., and Suntzeff, V. Influence of age on epidermal carcinogenesis induced by methylcholanthrene in mice. *Yale J. Biol. & Med.* 17: 47-58, 1944.
192. Cramer, W., and Stowell, R.E. The early stages of carcinogenesis by 20-methylcholanthrene in the skin of the mouse. I. Experimental technique and macroscopic changes. *J. Nat. Cancer Inst.* 2: 369-378, 1942.
193. Cramer, W., and Stowell, R.E. The early stages of carcinogenesis by 20-methylcholanthrene in the skin of the mouse. II. Microscopic tissue changes. *J. Nat. Cancer Inst.* 2: 379-402, 1942.
194. Dick, G.F., and Leiter, L. Some factors in the developments, localization and reabsorption of experimental amyloidosis in rabbits. *Am. J. Path.* 17: 741-753, 1941.

- 195.. Edwards, J.E., and Dalton, A.J. Induction of cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. *J. Nat. Cancer Inst.* 3: 19-41, 1942.
196. Eklung, C.H., and Reinam, H.J. The etiology of amyloid disease, with a note on experimental renal amyloidosis. *Arch. Path.* 21: 1-9, 1936.
197. Gardner, W.U. Testicular tumors in mice of several strains receiving triphenylethylene. *Cancer Research* 3: 92-99, 1943.
198. Green, H.H. An immunological concept of cancer: A preliminary report. *Brit. Med. J.* 2: 1374-1380, 1954.
199. Green, H.H. Immunological basis of carcinogenesis. *Brit. Med. Bull.* 14: 101-105, 1958.
200. Green, H.H. The immunological theory of cancer. *Acta Un. int. Cancr.* 17: 215-233, 1961.
201. Haddow, A. Cellular inhibition and the origin of cancer. *Acta Un. int. Cancr.* 3: 342-353, 1938.
202. Henshaw, P.S. Experimental Roentgen injury. IV. Effects of repeated small doses of x-rays on blood picture, tissue morphology, and life span in mice. *J. Nat. Cancer Inst.* 4: 513-522, 1944.
203. Jaffe, R.H. Amyloidosis produced by injection of proteins. *Arch. Path.* 1: 25-36, 1926.
204. Jones, R.S., and Frazier, D.B. Primary cardiovascular amyloidosis: Its clinical manifestations, pathology and histogenesis. *Arch. Path.* 50:366-384, 1950.

205. Khanolkar, V.R. Functional components of chemically induced mammary cancer. *Acta Un. int. Cancr.* 17: 45-54, 1961.
206. Kuczynski, M.H. Neue Beitrage zur Lehre von Amyloid. *Klin. Wchnschr.* 2: 727-730, 1923.
207. Landsteiner, K. The specificity of serological reactions. Chap. 5. Harvard University Press, Cambridge, Mass.
208. McCombs, R.P. Periarthritis nodosa and related disorders of blood vessels. *DM* pp. 1-31, 1960.
209. Maximow, A. Ueber die experimentell hervorgerufene Amyloid-Entartung der Leber. *Virchows Arch. f. Path. Anat.* 153: 353-401, 1898.
210. Miller, E.C., and Miller, J.A. The presence and significance of bound aminoazo dyes in the livers of rats fed by p-Dimethylaminoazobenzene. *Cancer Research* 7: 468-480, 1947.
211. Miller, E.C. Studies on the formation of protein-bound derivatives of 3,4-benzpyrene in the epidermal fraction of mouse skin. *Cancer Research* 11:100-108, 1951.
212. Miller, E.C., and Miller, J.A. In vivo combinations between carcinogens and tissue constituents and their possible role in carcinogenesis. *Cancer Research* 12: 547-556, 1952.
213. Nowak, J. Experimentelle Untersuchungen ueber die Actiologie der Amyloidosis. *Virchows Arch. f. Path. Anat.* 152: 162-190, 1898.

214. Orr, J.W. Changes antecedent to tumour formation during treatment of mouse skin with carcinogenic hydrocarbons. *J. Path. Bact.* 46: 495-515, 1938.
215. Peacock, P.R., and Beck, S. Rate of absorption of carcinogens and local tissue reaction as factors influencing carcinogenesis. *Brit. J. Exper. Path.* 19: 315-319, 1938.
216. Pease, H.D., and Pearce, R.H. Liver necrosis and venous thrombosis in horses actively immunized with diphtheria and tetanus toxins and with streptococci and their products. *J. Infect. Dis.* 3: 619-637, 1906.
217. Pullinger, B.D. The first effects of mouse skin of some polycyclic hydrocarbons. *J. Path. Bact.* 50: 463-471, 1940.
218. Rich, A.R. The role of hypersensitivity in periarteritis nodosa as indicated by seven cases developing during serum sickness and sulfonamide therapy. *Bull. Johns Hopkins Hosp.* 71: 123-140, 1942.
219. Rich, A.R., and Gregory, J.E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.* 72: 65-68, 1943..
220. Richter, G.W. The resorption of amyloid under experimental conditions. *Am. J. Path.* 30: 239-261, 1954.

221. Rigdon, R.M. Amyloidosis-Experimental production in ducks with methylcholanthrene. Texas Rep. Biol. Med. 18: 93-102, 1960.
222. Simpson, W.L., and Cramer, W. Fluorescence studies of carcinogens in skin; histological localization of 20-methylcholanthrene in mouse skin after single application. Cancer Research 3: 362-369, 1943.
223. Simpson, W.L., and Cramer, W. Fluorescence studies of carcinogens in skin; mouse skin after single and multiple applications of 20-methylcholanthrene. Cancer Research 5: 449-463, 1945.
224. Spencer, R.R. Carcinogenesis and cell adaptations. Cancer Research 6: 485, 1946.
225. Steiner, P.S. The nature of Cancer. Canadian Cancer Conference Vol. 1, 1955, pp. 74-90. Academic Press Inc., Publishers, N.Y.
226. Strong, L.C. A new influence on chemically induced sarcomata. Science 108: 688-689, 1948.
227. Tabachnick, M., Eisen, H.H., and Levine, B. A new mixed disulphide: Penicillamine-Cysteine. Nature 174: 701-702, 1954.
228. Teilum, G. Cortisone-ascorbic acid interaction and the pathogenesis of amyloidosis. Ann. Rheum. Dis. 11: 119-132, 1952.
229. Teilum, G. Studies on pathogenesis of amyloidosis. II. Effect of nitrogen mustard in inducing amyloidosis.

- J. Lab. Clin. Med. 43: 367-374, 1954.
230. Williams, M.G. The response of the human epidermis to the application of carcinogenic hydrocarbons. J. Invest. Dermatol. 30: 13-20, 1958.
231. Wilson, R.H., DeEads, F., and Cox, A.J. The carcinogenic activity of 2-acetaminofluorene. III. Manner of administration, age of animals, and type of diet. Cancer Research 7: 450-452, 1947.
232. Yamagawa, K., and Ichikawa, K. Experimental study of the pathogenesis of carcinoma. J. Cancer Research 3: 1-29, 1918.
233. Zeek, F.M. Periarteritis nodosa: a critical review. Am. J. Clin. Path. 22: 777-790, 1952.
234. Zeek, F.M. Periarteritis nodosa and other forms of necrotizing angiitis. New England J. Med. 248: 764-772, 1953.

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