HUMAN AND EXPERIMENTAL BREAST CANCER

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PREFACE

THIS book is concerned primarily with the aetiology, pathology and treatment of cancer of the human breast. It should be of interest to experimentalists, pathologists, surgeons and radiotherapists. The authors believe that the time has come when the knowledge gained from experiments in animals can be used directly in the study of the human disease, including the planning of therapy. The conception of the hormone-dependent breast cancers and the introduction of adrenalectomy as a form of therapy, were due to the application of principles derived from the experimental study of the prostate by Huggins and his colleagues.

Although it was not intended that this should be a treatise on the breast and its diseases, normal breast structure and function have inevitably been included. A detailed knowledge of the normal structure and of the factors concerned in the development, maintenance and involution of the mammary gland and in the control of its secretory functions are essential to the understanding of the modern endocrine therapy of breast cancer. Certain diseases of the breast other than cancer have also had to be considered: some such as cystic disease because they are precancerous; others such as duct ectasia because they throw light on the structure and function of the gland. Only aspects on which we have new information and new ideas are included. Thus, conditions such as granular cell myoblastoma, on which we have no fresh knowledge, have not been discussed. In view of this, the book is to some extent selective and because many new ideas are included, it is also controversial, for it is not to be expected that these ideas will gain immediate acceptance.

We are glad of the opportunity to acknowledge with gratitude the help we have received over a long period of years from many colleagues. The experimental material is derived from the Department of Experimental Pathology and Cancer Research, University of Leeds. For the clinical material we are indebted to Mr. H. S. Shucksmith, F.R.C.S., and to many surgeons and radiotherapists of the Leeds General Infirmary and St. James's Hospital, Leeds. The photographs are largely the work of Mr. C. N. England, University of Leeds, and Mr. J. Hainsworth, St. James's Hospital, Leeds. We thank Dr. D. B. Clayson and Miss Valerie Simpson for much help in the preparation of the manuscript.

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INTRODUCTION

THE fundamental experimental studies already made concerning the factors involved in the genesis of cancer in general, and of cancer of the breast in particular, have a profound bearing upon the study of the human disease. The question immediately arises as to how far the explanations offered by animal experimentation can be applied to human cancer. It has to be accepted that only rarely can the experimental method be applied in the human and that laboratory animals must therefore be used in substitute. The choice of the experimental animal is dependent upon the problem to be investigated but, for practical and economic reasons, small rodents such as mice, rats and hamsters are the animals of choice for cancer investigations. The advantages of experiments in animals are threefold: (1) selected factors can be kept constant while others are varied at will, so that the role of an individual factor can be judged; (2) many animals can be kept in identical experimental conditions and thus it can be decided whether an observed result is due to chance or is reproducible; and (3) animals which have established characteristics can be chosen so that any departure from their usual behaviour is readily noticed. For this purpose animals of inbred strains are valuable because the variation in individual response to experimental procedures is kept to a minimum.

The disadvantage of animal experimentation is that the results observed in one species may not necessarily be reproduced exactly in another. Although this makes the direct application of an experimental result to the human disease impossible, the study of species differences is of just as great value as the study of species similarities. For example, it has been possible on one occasion to correlate species differences in the method of metabolism of a carcinogenic agent with differences in the site and incidence of the induced tumours.

It was established early in the century that mouse mammary cancer is a true malignant disease, and, in about 1930, American workers established the first inbred strains of mice in which the spontaneous incidence of breast cancer varied from nothing to 100 per cent in breeding females. Inbred strains are obtained by brother-sister matings until all the individuals are genetically identical and their genetic constitution can change only by mutation. In mice, at least twenty generations of inbreeding are required before we can speak of an inbred strain. Even after this degree of inbreeding it is necessary to maintain brother-sister matings and to avoid the development of sub-lines within the strain by breeding only from the central trunk of the breeding tree.

The number of inbred strains and the countries from which they come has increased in

the succeeding years. They have specific reactions and characteristics which are well known and are of great use in planning experiments concerned with, for example, hormone production. It is necessary to have a working knowledge of the main characters of these strains in order to interpret their reactions to various treatments. It is also necessary to understand their nomenclature. The name of the strain has usually been decided by the worker who carried out the original selection and there is thus a bewildering number of abbreviations or combinations of letters and numbers without order or meaning. Examples of these are the strains C3H, dba, A, 0·20 and IF.

In mammary cancer work it is essential to know whether or not a strain carries the mammary tumour agent or milk factor. This has the characteristics of a virus and is transmitted by the mother's milk to the offspring by suckling. Once a mouse receives the agent, it remains infected with it for life. As this is an extremely important factor in the study of mouse mammary cancer, its absence is represented by the addition of "b," "e" or "f," according to the method by which it was eliminated from the strain. Occasionally, in the older literature, absence of the agent is denoted by "x." If the experimental work which has been done on these strains is to be understood, it is necessary to become accustomed to this nomenclature. Mice of pure strains are often hybridized to give, for example, IF x A hybrids. Such offspring may or may not possess the mammary tumour agent, depending on its presence or absence in the mother.

Inbred strains, both of mice and of rats, are valuable not only in the study of spontaneous mammary cancer but are necessary in order to study the factors concerned in the planned induction of the disease by various means. From such studies it has become clear that we already have three methods of inducing mammary cancer in rodents: (1) hyperhormonal stimulation; (2) the combined action of the mammary tumour agent, hormones and genetic constitution; and (3) the use of chemical carcinogens. Not only are the factors concerned in the induction of these tumours partially unravelled, but also histological types characteristic of the mode of induction are emerging, and these give hope that similar patterns will be discernible in the human disease. A large field awaiting study is a comparison of the preneoplastic lesions of mouse and man. There are many points of similarity in the evolution of mammary cancer in the two species; in both the origin is frequently multicentric and in both the process is often a gradual one, to be seen at different stages in the same breast or in different breasts in the same subject. It is not possible to state exactly at what stage cells acquire the malignant character, but in both species it is before the break-through into the tissue spaces. A later chapter of this book is devoted to a consideration of the evidence which demonstrates that cystic disease is a frequent precursor of mammary cancer in the human. Similar studies in animals are lacking but could be pursued both in the mouse and in the rabbit.

It should not be thought that morbid anatomical studies have outgrown their usefulness in unravelling the aetiology and pathology of breast diseases. The interpretation of all experimental work is dependent upon a correct assessment of the pathological nature of the induced lesions, and the value of the experimental results can be greatly enhanced by discriminating morphological studies. With these facts in mind the morbid anatomical studies which are described in the chapters on human breast disease in this book were undertaken.

The aim was to correlate what was seen with the naked eye and under the microscope with the information which could be obtained of previous breast disease, parity, hormonal status, etc. These studies have yielded results which surpass the expectations held at their initiation.

Statistical investigations have been used by many workers to correlate the incidence of human breast cancer with factors such as age and hormonal status, the latter modified by child bearing and the menopause. Frequently these studies would be more valuable if all the information were reported in a more detailed manner and if the full reproductive history of all the patients were accurately known. It is too often tacitly assumed that carcinoma of the human breast is a simple entity irrespective of whether it arises in a nulliparous woman of 90 or in a woman of 30 with six children. From analysis of the relevant data it may eventually prove possible to fit the established methods of treatment more effectively to the individual case.

Statistical evaluation of the results of any form of therapy, whether surgical, hormonal or chemical, is essential to determine whether any particular therapeutic régime does in fact produce a real improvement in the condition of the subjects. Such observations must be related to the spontaneous variations, of unknown origin, which may be observed in a random series of patients. Statistical methods are nevertheless only one form of assessing the effects of any measure. Ethically and scientifically the individual person with breast cancer is more than a unit necessary in the collation of a significant result. Variations in the course of cancer progression in a single subject may indicate factors of fundamental importance in the development of discriminating therapy.

The ultimate object of animal experiments must be to apply the information obtained to the treatment of cancer in man. This logical objective is inclined to arouse considerable antagonism, on the grounds that experiments in animals cannot be applied to the human problem. Such objections are not limited to non-scientists but must surely be irrational. The nature of disease processes in animals has been used throughout the history of medical research to throw light on the aetiology and treatment of human conditions. In the absence of experiments in humans it is the only possible biological approach, and it is a matter of fact that the frequently empirical attempts to control disease, based on animal observations, have provided the starting point for most of the major contributions to medical progress. It would be short-sighted to assume that the results of animal experiments can *necessarily* be applied directly to the human without modification, but in order to see to what extent conditions in animals are comparable with conditions in man, attempts to interpret one in terms of the other must be made.

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CHAPTER ONE

Factors Concerned in the Induction and Growth of Cancer in General

SINCE the beginning of this century the volume of fundamental research work pertaining to the study of cancer has increased very greatly. This book is devoted to cancer of the breast and it is therefore fortunate that a large proportion of cancer research has been concerned with this organ. None the less, much has been learned of the behaviour and nature of malignant disease in general from work concerned primarily with other tissues and it is this general knowledge that will be considered in this chapter.

DEFINITION OF CANCER

Cancer may be defined as the multiplication of a specific tissue in excess of the requirements of the body and persisting even when the stimulus which provoked it has been removed, although growth may still be dependent on other factors such as hormones. Such growth is *malignant* in that the component cells infiltrate the enveloping tissues and ultimately may be carried by lymphatic and haemic transport to sites remote from that of origin, where they constitute new foci of metastatic tumour. This uninhibited cancerous growth usually proceeds at such a pace as to cause the premature death of the host, primarily by the physical obliteration and impairment of essential organs, and secondly by the toxic action of the products of necrosis. It is also possible that a haemolytic factor may be produced specifically by the tumour cells. When the cancer cells are derived from a glandular epithelium and have retained their secretory function, the host may exhibit the symptoms of hyperfunction of the organ in question; for example hyperthyroidism in the case of secreting carcinomas of the thyroid.

It is frequently difficult to determine microscopically whether a lesion should be classed as cancerous or not, and it will be seen from the contents of this chapter that the definition adopted is open to qualification in some respects. *Benign* hyperplastic changes which anatomically resemble early cancer occur, but these do not persist when the provoking stimulus is removed. They do not invade the enveloping tissues and never form the focus of a new growth at a site distant from that of their origin.

The reader will be familiar with the commonly-used nomenclature of cancer (Willis, 1953), the term *carcinoma* denoting a neoplasm arising in epithelial tissue and *sarcoma* one arising

in connective tissue. Either term may be qualified by a prefix descriptive of the form, function or components of the particular tumour. The term *neoplastic change* will be used to denote a train of events leading to the emergence of cancer.

AGENTS CAUSING CANCER (CARCINOGENS)

Neoplasia may be induced by a variety of agents, some of which affect all the tissues exposed to them, and some of which are more or less specific for certain types of tissue or organs.

Chemical Carcinogens

It has long been known that exposure to certain substances is associated with an increased risk of developing cancer. The first record of such an observation was that of Pott (1775) who described scrotal cancer caused by soot in chimney sweeps. Similarly the association of cancer with tar, mineral oils, and dyes and their intermediates became apparent from clinical observation and was confirmed by experimental evidence, which is reviewed by Hueper (1957). Induction of cancer was achieved experimentally in 1915 (Yamagiwa and Ichikawa, 1918) who induced skin cancer in rabbits by the repeated application of coal tar. This observation, soon confirmed by other workers, was of fundamental importance in that it provided a means of devising controlled experiments and led to the identification and synthesis of the large class of polycyclic hydrocarbons which are potent cancer-inducing substances. The first pure chemical carcinogen, 1 : 2 : 5 : 6-dibenzanthracene (XIV)[1], was obtained synthetically by Kennaway and his co-workers, and this advance was soon followed by the identification of the carcinogen 3 : 4-benzpyrene (XIII) in coal tar. The early investigations of chemical carcinogens have been reviewed by Kennaway (1955). The present state of knowledge of these important agents and their mode of action has been summarized by Haddow (1958). Of this group of chemicals, the most important in the study of breast cancer have proved to be: 20-methylcholanthrene (XI), 1 : 2 : 5 : 6-dibenzanthracene, 3 : 4-benzpyrene and 9 : 10-dimethyl-1 : 2-benzanthracene (XII).

The demonstration by Yoshida (1932) that the dye o-aminoazotoluene caused liver tumours when fed to rats, led to the discovery of two other classes of carcinogens. Certain azo dyes such as 4-dimethyl-aminoazo-benzene (butter yellow), 1-phenylazo-2-naphthol (oil orange E, XXV) and o-tolylazo-2-naphthol (oil orange TX, XXVI) have since been shown to be carcinogenic, as they induce tumours of the liver and intestine of mice. In a recent publication (Bonser, Clayson and Jull, 1956) evidence was presented that other azo dyes have similar activity, although in lesser degree. More thorough testing of compounds of this class is urgently needed as their industrial use is widespread and azo dyes of the same type are in use as food colourants.

The aromatic amines, from which azo dyes are derived, were for a long time suspected of cancer-inducing properties. As early as 1895 Rehn had found an increased incidence of bladder cancer among workers making magenta. Despite many attempts, however, it was not until 1938 that Hueper, Wiley and Wolfe were able to induce cancer of the bladder in dogs experimentally by feeding 2-naphthylamine (XVI), a substance in common use as a dye intermediate in the dyestuffs industry. His work was confirmed by Bonser (1943) and

[1] Throughout the book these roman numerals denote the chemical formulae given in the Appendix.

since then convincing evidence has been produced to show that the effective agent in this form of cancer induction is in fact a metabolic product of 2-naphthylamine, i.e. 2-amino-1-naphthol (XVII). This discovery leads to the conclusion that exogenous chemicals are capable of alteration by normal bodily processes to carcinogenic substances. Furthermore, it has been shown that the metabolites so formed attain different concentrations in different sites. For example, in the dog, the ratio of concentration of 2-amino-1-naphthol in urine and serum was 200 : 1, a fact which would account for the occurrence of tumours of the bladder in this species following oral administration of 2-naphthylamine. Because of the technical difficulties involved, no attempt has yet been made to demonstrate metabolic differences in other organs and excretions.

Many ubiquitous carcinogens have since been discovered among the related aromatic amines. 2-Acetamidofluorene (XXIII), first investigated by Wilson, DeEds and Cox (1941), was shown to induce cancer in many sites by different modes of administration. That this activity was discovered by toxicity tests was indeed fortunate as its use as an insecticidal crop spray was planned. Benzidine (XIX) and derivatives of 4-aminodiphenyl (XVIII) and 4-aminostilbene (XX) have also been shown to produce tumours in a variety of tissues. Badger and Lewis (1952) excellently reviewed the work in this field and Clayson (1953) has put forward a hypothesis, covering the aromatic amines generally, which attempts to classify them according to chemical structure. The aromatic amines which are known to induce cancer of the breast are: 2-acetamidofluorene, 4-aminodiphenyl, 4-aminostilbene and many of their substituted derivatives.

In addition to the compounds which have been discussed so far, and which fall into groups of similar chemical structure, there are various apparently unrelated substances for which carcinogenic activity has been claimed. These are all listed by Hartwell (1951) and the experimental evidence is also summarized. It should be borne in mind that this invaluable publication does not act as a judge of carcinogenic activity, but only as a record of experimental fact. When it is consulted the reader should critically evaluate the evidence by reference, where necessary, to the original papers. When compounds are tested for cancer-inducing activity the results, whether apparently positive or negative, must be assessed, bearing in mind the following factors: the size and frequency of the dose, the mode of administration, the duration of the experiment, the species, sex and strain of the experimental animal, the normal incidence of cancer in that animal, the diet, environmental factors such as the nature of the cage accommodation and the temperature, and not least the extent and intensity of the post-mortem examination. When such a critical examination of these variables is made it is sometimes found that the evidence of cancer induction is unconvincing, or could be explained by coincident factors not related to the compound under test. In this connexion it may be noted that arsenic is sometimes loosely referred to as a carcinogen, and yet the evidence on which the statement is largely based is only fragmentary (Neubauer, 1947), and was never claimed to be more than suggestive. Conversely, compounds which are generally regarded as not carcinogenic may not have been sufficiently tested.

Of the miscellaneous carcinogenic chemicals, one of the most interesting is urethane. This was originally observed to act in a limited manner, in that it merely caused an increase (although often a considerable increase) in the incidence of lung adenomas in mice. Recently,

however, it has been shown to be carcinogenic when applied to mouse skin, if the treated area is subsequently painted with croton oil. This phenomenon of "co-carcinogenesis" will be discussed more fully later in this chapter.

Viruses

It has been and still is maintained by some authorities that infection by a virus is responsible for all forms of cancer. This extreme view is countered by an equally vehement body of opinion which maintains that no form of cancer is caused by a virus. Before considering briefly what role viruses have been shown to play in carcinogenesis, it is well to consider what a virus is. Unfortunately no clear definition can be stated but viruses do have several general characteristics in common. They are sub-microscopic protein complexes capable of passing through a filter which will hold back the smallest bacteria. They are capable of self-reproduction to an apparently unlimited extent, but only in the presence of intact living cells. Individual virus forms are highly specific in their growth requirements and, thus, in the pathological symptoms they induce in a given host. Mutations are common and thus sudden variations in pathogenic activity are frequently observed even with the same virus strain.

To support any suggestion that a viral agent is the active factor in cancer induction it is absolutely necessary to show that cell-free extracts, obtained by ultra-filtration or centrifugation, can give rise to the disease when injected into test animals. Failure to conform with this fundamental condition has been responsible for a number of claims that a virus was responsible for the production of cancer. There are, nevertheless, several types of cancer in which the role of viruses as the causal factor has been established beyond doubt (Oberling and Guérin, 1954).

A fibromyxosarcoma in a hen reported by Rous (1910, 1911) was undoubtedly induced by a virus and many sarcomatous fowl tumours of a more or less similar type have been reported (Foulds, 1934). These bird tumours are characterized by their extremely rapid appearance, forming quickly-growing nodules two or three weeks after the injection of cell-free tumour extracts. It should be noted that there is no substantiated case of a chemically-induced fowl sarcoma which is transmissible by a cell-free extract (Peacock, 1946) and, therefore, although some fowl sarcomas are caused by virus infection, others can be induced by chemical or physical agents. Various erythroblastoses and lymphomatoses in birds have also been shown to be due to viruses.

A renal tumour of the leopard frog, discovered by Lucké (1934 a, b), when transplanted subcutaneously into other frogs did not grow locally but induced tumours in the kidneys of the hosts. That these were true cancers was shown by the fact that they metastasized (Lucké, 1938 a, b).

Shope (1933) described multiple papillomatosis of the skin in the cotton-tail rabbit certainly caused by a virus. When transferred to the domestic rabbit this agent also produced multiple epithelial tumours, 75 per cent of which became malignant in four to seven months. This virus provides an interesting example of adaptation to the environment, as it withstands heating to 65° C for 35 minutes. This property is important in the natural desert conditions, where the virus is spread by the shedding of the warty epithelial masses from infected animals, an environment in which it can survive for a long time despite the hot climate. The Shope

papilloma virus played an important role in early studies on the mechanism of cancer induction, for it was shown by Rous and Kidd (1938) that if the skin of rabbits was tarred, and they were subsequently infected with this agent, skin tumours appeared and became malignant more quickly than in rabbits which received tar alone. This phenomenon is readily explicable on the basis of the two-stage mechanism of "initiation" and "promotion" of skin tumours which was originated by Rous and his collaborators and further developed by Berenblum and others (p. 9).

The viral agents so far discussed are characterized by the fact that they must be applied directly to the tissue they affect and are not effective by intravenous injection. In the Shope papilloma virus the agent must come into contact with the basal layers of the skin so that epithelial damage is a prerequisite for successful infection. These agents readily bind to protein and this probably accounts for the necessity for their direct application. It is interesting and probably very significant that in the protein-bound state the virus is highly resistant to irradiation, by which it is rapidly destroyed when pure.

Recently (Gross, 1953 a, b, c) it has been reported that mouse leukaemia is also transmissible by cell-free extracts from the Ak strain, which has a high natural incidence, to the C3H strain which has a low incidence. The mice of the recipient strain develop the disease one to two years after inoculation with filtered extracts of organs of the donor strain. The leukaemia so induced was transplantable within the C3H strain but cells could not be grafted back into the Ak strain. This is supporting evidence for the conclusion that the disease had been induced in the tissues of the C3H mice by a virus-like factor. Furthermore, the progeny of the infected C3H mice had a leukaemia incidence of 48 per cent.

An added interest of this agent of mouse leukaemia is the fact that diluted cell-free extracts induce salivary-gland tumours as well as leukaemia in newborn mice of the C3H strain (Gross, 1953 b). It is thus possible that there is more than one effective agent in these preparations from the tissues of leukaemic mice.

The viral agent, associated with carcinogenesis, which has been most intensively investigated is the mouse mammary-tumour agent, first demonstrated by Bittner in 1936. There now seems little doubt that this agent should be classified as a virus, although this point has been disputed from time to time. The milk agent is present in the tissues of many strains of mice and has also been demonstrated in wild mice (Andervont and Dunn, 1956). There are, however, some strains of inbred mice, notably the C57 black and IF strains, in which there is no evidence at all that this factor is present in any form, although they may be infected with it artificially. Thus, although of paramount importance in the study of mammary carcinogenesis in the mouse, the mammary tumour agent should not be regarded as the sole initiator of breast tumours in this species.

This virus is present in a highly active form in the milk of infected mice and in this manner is passed on to their offspring, which in turn become infected and act as a source of infection for further generations. Mice which have once been infected by the agent can never themselves be freed from it and provided their hormonal environment is suitable they will be liable to develop breast cancer. The agent-induced mammary-tumour incidence varies in different inbred strains of mice (0-100%) according to a number of factors, some of which have been very thoroughly investigated. The results of these investigations will be discussed

more fully in Chapter 7. All the available information on this important subject has recently been reviewed by Dmochowski (1957).

It seems that the milk agent is unique in its action on the mouse breast. There has been, to date, no evidence of a similar agent in rats, although the latter often have a high incidence of spontaneous mammary cancer. Investigations have so far failed to produce any evidence of a comparable agent in human breast cancer.

Hormonal

In the normal intact animal the pituitary secretes tropic hormones which stimulate growth and secretion by the other endocrine glands (Chapter 2). Under physiological conditions the secretion of tropic pituitary hormones is inhibited by the action on the pituitary of the hormones whose secretion they stimulate. The level of any particular pituitary hormone is, therefore, inversely proportional to that of the hormone secreted by its target organ, allowing for the time-lag required for the increased amounts of pituitary-stimulated secretion to exert their suppressive action on the pituitary.

Pituitary FSH (follicle stimulating hormone) stimulates secretion of oestrogen by the ovary. However, if exposure of the pituitary to the increased concentration of oestrogen is prevented, FSH concentration will tend to increase and to be maintained at a high level. Biskind and Biskind (1944) demonstrated that if bilateral ovariectomy was performed in the rat and one ovary was transplanted beneath the splenic capsule, the oestrogens produced by that ovary were carried directly to the liver and immediately de-activated. The animals so treated were in constant anoestrus. If, by accident, adhesions occurred between the intrasplenic graft and the abdominal wall, oestrogen could escape into the peripheral circulation and exhibit its normal physiological action, as evidenced by vaginal keratinization.

It was found that when operation was satisfactory and the animals remained anoestrous, the ovarian grafts became hyperplastic and eventually formed relatively benign tumours. Some of these tumours progressed to malignancy, which was at first hormone-dependent. If the life of the graft were prolonged by successive transfer to castrated rats of the same stock, the hormone-dependence of the tumours was gradually lost and they became increasingly malignant in character. This development of a malignant tumour is attributable to the continued hyperstimulation of the ovarian graft by elevated concentrations of pituitary FSH.

Similar examples of carcinoma induction in other tissues are well known. Of these, the occurrence of adrenal cortical carcinomas after castration in certain strains of mice has been extensively investigated (Woolley, 1950). These adrenal cortical tumours usually secrete large quantities of sex hormones, including oestrogens, the effect of these being to counterbalance the removal of the gonads. The occurrence of these changes is attributable to hyperstimulation by pituitary secretions as a consequence of removing the inhibitory action of the ovarian or testicular hormones.

Conversely, tumours of the pituitary gland itself may be caused by excessive stimulation of that organ to produce tropic hormones. Such pituitary stimulation may be induced by the removal of the thyroid, which causes an uninhibited stimulation of TSH (thyroid stimulating hormone), or the administration of continued high levels of oestrogen, which results in excessive prolactin secretion. Such pituitary tumours are composed of the cell type respon-

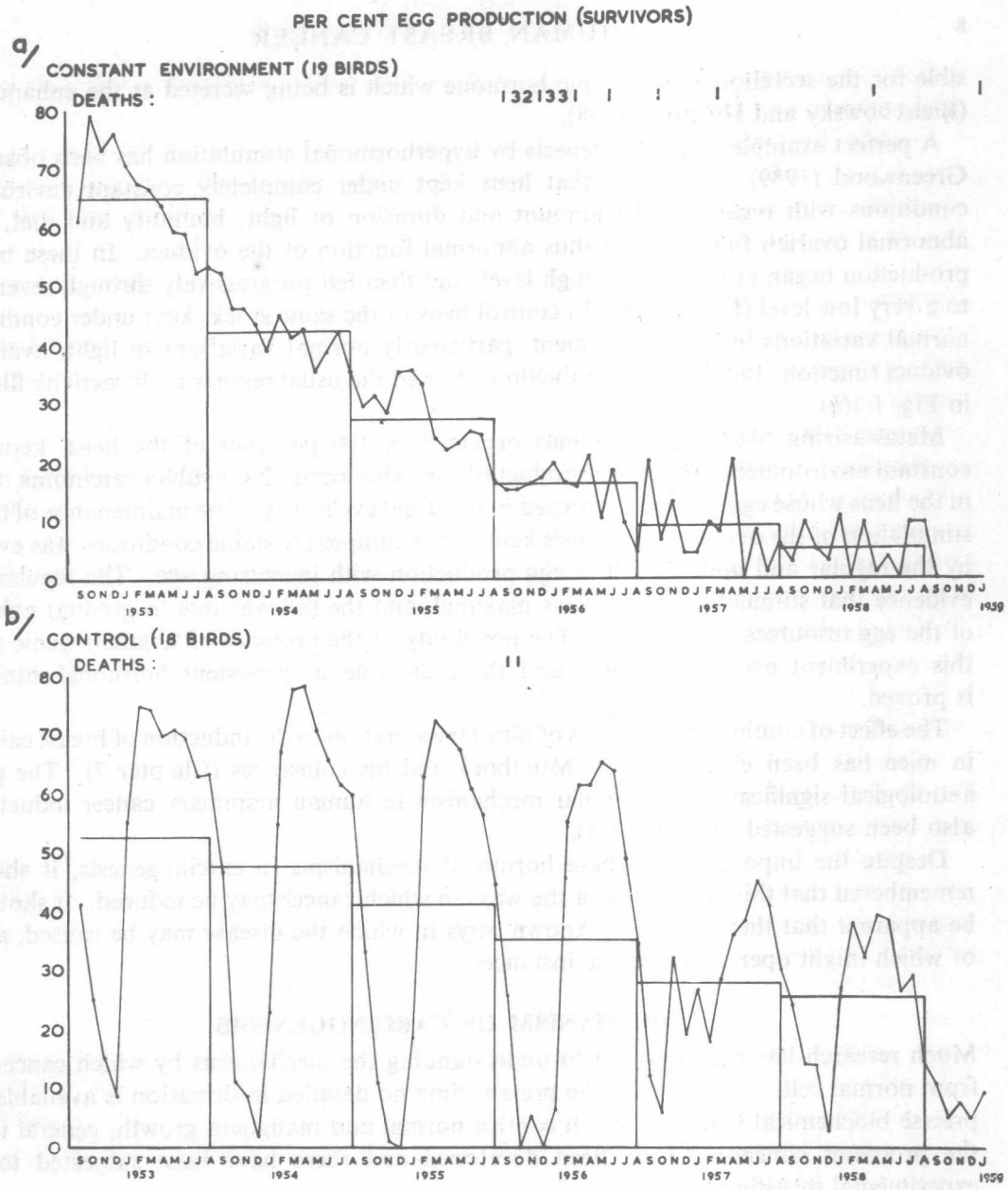

FIG. 1.1. VARIATIONS IN MONTHLY EGG PRODUCTION IN TWO GROUPS OF POULTRY

(*a*) When kept in constant conditions of temperature, humidity and light.
(*b*) When kept in a normal environment.

(*By courtesy of* Dr. A. Greenwood)

sible for the secretion of the tropic hormone which is being secreted at the enhanced level (Bielschowsky and Horning, 1958).

A perfect example of carcinogenesis by hyperhormonal stimulation has been observed by Greenwood (1959). He found that hens kept under completely constant environmental conditions with regard to the amount and duration of light, humidity and diet, had an abnormal ovarian function and thus abnormal function of the oviduct. In these birds egg production began at a relatively high level, and then fell progressively through several years to a very low level (Fig. 1.1(*a*)). In control hens of the same stock, kept under conditions of normal variations in the environment, particularly normal variations in light, ovarian and oviduct function, and thus egg production, showed the usual regular cyclic activity illustrated in Fig. 1.1(*b*).

Metastasizing oviduct[1] carcinomas occurred in 100 per cent of the hens, kept in the constant environment, whose egg production was abnormal. No oviduct carcinoma occurred in the hens whose egg production varied in the usual cyclic way. The maintenance of constant stimulation of the oviduct in the birds kept under completely stable conditions was evidenced by the regular and uniform fall in egg production with increasing age. The regularity was evidence that stimulation was always maximal, and the fall was due to gradual exhaustion of the egg resources of the ovary. The possibility of the presence of a carcinogenic agent in this experiment can be excluded, and thus, the role of persistent hormonal stimulation is proved.

The effect of continued high levels of pituitary secretion in the induction of breast carcinoma in mice has been established by Mühlbock and his colleagues (Chapter 7). The possible aetiological significance of a similar mechanism in human mammary cancer induction has also been suggested by Jull (1958).

Despite the importance of these hormonal mechanisms in carcinogenesis, it should be remembered that this is only *one* of the ways in which cancer may be induced. It should now be apparent that there are many known ways in which the disease may be caused, any one of which might operate in specific instances.

MECHANISM OF CARCINOGENESIS

Much research has been devoted to understanding the mechanisms by which cancers arise from normal cells. Although at the present time no detailed information is available of the precise biochemical reactions which control normal and malignant growth, general ideas of the processes concerned have been developed and these have been subjected to much experimental investigation.

In 1923 Deelman claimed that scarification of mouse skin before the application of tar speeded the appearance of tumours. His observations were not generally confirmed (Ludford, 1929) but it was established that tar tumours in the mouse are preferentially located at the edges of deep incisions. This localization was also demonstrated following the use of benzpyrene in the mouse (Pullinger, 1943). Other carcinogens in place of tar gave varying results (Berenblum, 1954).

[1] Until a full histological examination has been made, the origin of these carcinomas, whether from ovary or oviduct. is open to doubt. Greenwood has not yet published a full report.