

Hormones and neoplasia

A. P. M. FORREST

From the Department of Surgery, University of Edinburgh

My task is to give a broad and general review of the role of hormones in experimental and human neoplasia. Since hormones are concerned with the growth of all tissues, this is possible only if one considers specific hormones and their effect on specific tissues. This review is therefore limited to hormonal influence in cancer of the breast.

A Phenomenon

A phenomenon, the explanation of which still eludes us, is the regression of human breast cancer which may follow alterations in the hormonal environment. It was first observed in 1895 when recurrent cancer of the breast in a 33-year-old Glasgow woman remitted following removal of the ovaries (Beatson, 1896). The therapeutic advances which followed this observation are well known and include the administration of androgens and oestrogens and removal of the adrenals and the pituitary (Loeser, 1938; Ulrich, 1939; Haddow, Watkinson, and Paterson, 1944; Huggins and Bergenstal, 1952; Luft, Olivecrona, and Sjögren, 1952). The remission rates achieved by these various methods of treatment are remarkably similar; approximately one third of tumours have a beneficial response. Although in most patients the effects are short lived, in some they are prolonged and well nigh dramatic.

Normal Development of the Breast

It was accepted for many years that only the ovarian hormones, oestrogen and progesterone, were concerned with normal growth of the breast. It is now appreciated that even the simplest form of growth, that of the duct system, cannot be stimulated in the absence of hormones of anterior pituitary origin. The formation of lobules, of alveoli and the production of milk demand even more complex hormonal influences.

As a result of studies in hypophysectomized, oophorectomized, and adrenalectomized rats these are now more clearly defined (Lyons, 1958; Lyons and Dixon, 1966; Cowie and Tindall, 1971). The minimal requirements for ductal growth are now

considered to be oestrogen, adrenocortical steroids, and growth hormone. For full lobulo-alveolar development, equivalent to that at the termination of pregnancy, oestrogen, progesterone, prolactin, and growth hormone; and for the initiation of lactation, prolactin, growth hormone, and cortisol. In effect all the hormones shown in fig 1 have a part to play in normal growth of the breast.

Although species differences almost certainly occur it is reasonable to assume that the mechanism of growth control of the human female breast is similar. Small wonder the difficulty in defining the role of hormones on breast neoplasia, particularly as hormones are only one of several factors involved in the initiation and promotion of a tumour.

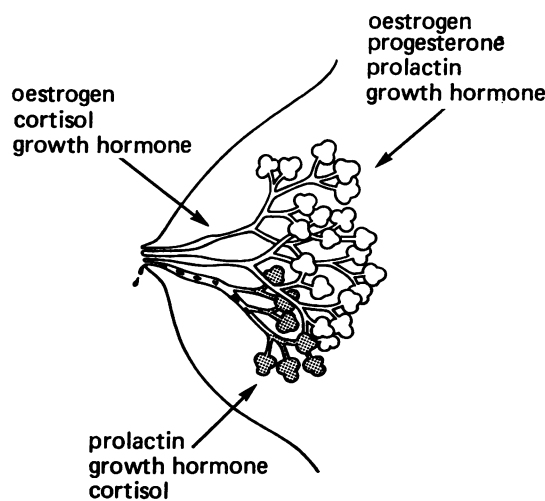


Fig 1 *Hormones concerned with normal breast development.*

Experimental Tumours

Since the studies by Mackenzie and Rous (1941) in tar warts, it has been accepted that there are two processes concerned with neoplasia: 'initiation' or the initial neoplastic transformation of a cell and

'promotion', the provision of conditions necessary for its continued growth and multiplication.

Apparently hormones can form the stimulus necessary for initiation or neoplastic transformation but only when given in unphysiologically large doses (Shimkin, 1958). Their principal effect is to act as promoting agents. Yet there is overlap, for a suitable hormonal environment may still be a necessary prerequisite for tumour induction, irrespective of the stimulus. For example, removal of the source of oestrogens in female rats, before the administration of 7, 12-dimethylbenzanthracene (DMBA), prevents tumour induction (Huggins, Briziarelli, and Sutton, 1959). Conversely, intact male rats, normally refractory to the effects of DMBA, develop mammary tumours when provided with functioning ovarian grafts (Dao and Greiner, 1961).

Early observations on spontaneous mammary cancer in mice, ie, that occurring in animals carrying the Bittner milk agent, indicated that the ovarian hormone oestrogen had a promoting role. Removal of the ovaries of female mice reduced its incidence whereas the grafting of functioning ovarian tissue or the administration of oestrogen to male mice, which normally do not develop spontaneous breast cancer, promoted tumour development (Lathrop and Loeb, 1916; Murray, 1928; Lacassagne, 1932). Similar effects have been observed with carcinogen-induced tumours (Bonser, Dossett, and Jull, 1961). There is evidence that the ovarian secretion of progesterone may also promote tumour development and when administered with oestrogen will further enhance its effect (Jull, 1954; Marchant, 1959; Bonser *et al*, 1961).

Spontaneous mammary cancer in mice may also be influenced by pituitary hormones. The implantation of extra pituitaries, which by virtue of their separation from hypothalamic control secrete prolactin, or the induction of prolactin-secreting tumours of the pituitary by irradiation, increase its incidence (Loeb and Kirtz, 1939; Furth and Clifton, 1958). Conversely hypophysectomy reduces it (Korteweg and Thomas, 1939). Evidence that pituitary hormones are necessary for the promoting effect of oestrogens comes from the observation that hypophysectomy abolishes the high incidence of mammary carcinoma in oestrogen-treated mice, even if oestrogen treatment is continued after the operation (Lacassagne and Chamorro, 1939). Forced breeding and pregnancies may also enhance the development of spontaneous mammary cancer in mice (Bonser *et al*, 1961).

Recently emphasis has moved from mouse to rat as an experimental model, using the tumour aptly named after Huggins. These mammary tumours can most simply be induced by a single intragastric or

intravenous pulse dose of DMBA in fat emulsion administered when the animal is 50 days of age. In suitable strains of Sprague-Dawley rats adenocarcinomas will develop in the majority of animals in 60 to 100 days. As most of these tumours have the unique property of hormone dependence they are particularly suitable for studies of hormonal effects.

It is firmly established that oestrogens affect the growth of Huggins tumour and some believe that the tumour is primarily oestrogen dependent (Dao, 1962). Removal of ovarian hormones by oophorectomy can induce regression, an effect which is reversible by their administration (Huggins *et al*, 1959; Sydnor and Cockrell, 1963; Young, Baker, and Helfenstein, 1965). Regression of tumours also follows the administration of testosterone or 5-alpha-dihydrotestosterone, this effect also being reversed by oestrogen and progesterone (Huggins *et al*, 1959; Young *et al*, 1965). The demonstration that oestrogen-receptor protein is present in the cells of the tumour and is related to their hormone sensitivity is further evidence that oestrogen is the prime hormone concerned (King, 1968; McGuire and Julian, 1971).

Yet there is now a growing belief that prolactin is equally, if not primarily, implicated. The induction of a high rate of prolactin secretion increases both their incidence and rate of growth (Clemens, Welsch, and Meites, 1968; Pearson, Llerena, Llerena, Molina, and Butler, 1969; Nagasawa and Meites, 1970). As prolactin secretion from the pituitary is controlled by a hypothalamic inhibitory factor, hyperprolactinaemia can readily be achieved by lesions of the median eminence of the hypothalamus, by hypothalamic oestrogen implants, or by the administration of the phenothiazine group of drugs. It has also been shown that the administration of antiprolactin, prepared by immunization, will induce tumour regression (Butler and Pearson, 1972). Recently we have had an opportunity to study three strains of rats in our laboratory which have different incidences of mammary tumour following a single intravenous injection of 5 mg DMBA. Blood was collected during dioestrus from 11 rats in each strain and the concentration of prolactin estimated by radioimmunoassay (Boyns, Buchan, Cole, Forrest, and Griffiths, 1973). This was clearly correlated with tumour incidence (fig 2).

The relationship between prolactin and oestrogen in stimulating tumour growth is further complicated by the finding that oestrogen, in all doses, stimulates prolactin secretion in the rat (Welsch and Meites, 1969). A similar effect occurs in the human female. In recent studies we have shown that both oral and intravenous oestrogen therapy induces hypersecretion of prolactin in women with cancer of the breast

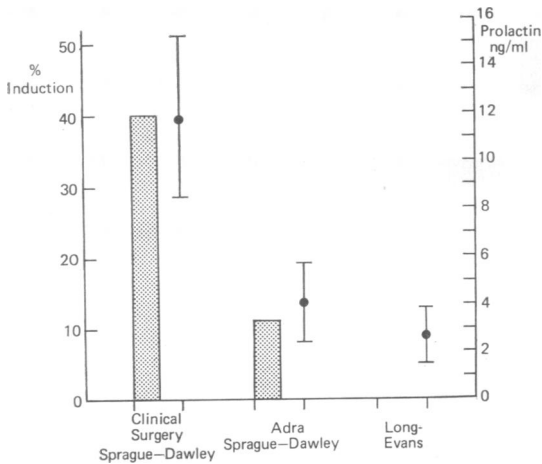


Fig 2 Circulating levels of prolactin and the incidence of mammary cancer induced by 7, 12-dimethyl benzanthracene in three strains of laboratory rats.

(Wilson, Buchan, Roberts, Forrest, Boyns, Cole, and Griffiths, 1973; fig 3).

These various studies with experimental tumours indicate that oestrogen, progesterone, and prolactin are three hormones concerned with the growth of experimental mammary cancer. Doubtless others may also be implicated.

Human Breast Cancer

Ovarian hormones also influence the behaviour of human breast cancer. The two factors known to

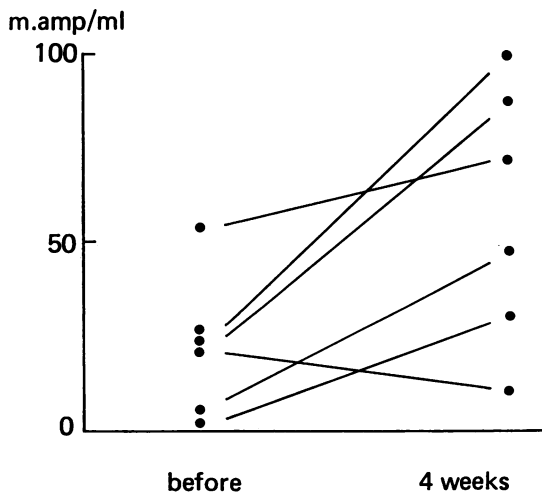


Fig 3 Effect of oral therapy with stilboestrol 5 mg tid on circulating prolactin levels in the human female.

exert greatest protection of a woman against the development of cancer of the breast are the age at first pregnancy and the previous performance of an oophorectomy (MacMahon, List, and Eisenberg, 1968; Lowe and MacMahon, 1970; MacMahon *et al*, 1970); regression of advanced breast cancer may occur during the natural menopause (Hadfield and Holt, 1956). Conversely enhancement of tumour growth has been observed during the ovarian cycle or as a result of the administration of oestrogen. Increase in the size of soft tissue lesions and of pain and hypercalciuria from bone metastases has been observed during the premenstrual period (Raven, 1950; Kennedy, 1956; fig 4).

Despite these observations, studies of circulating oestrogens have so far proved disappointing. Although the original observation of Huggins and Dao (1953) suggested that the urinary excretion levels of oestrogens, estimated biologically, were correlated with the clinical response to adrenalectomy, this has not been confirmed in subsequent studies using chemical assays (reviewed by Forrest, 1972). Minor differences in the form in which oestrogen is excreted have been noted in women with established breast cancer or who racially are at risk from the disease (Brown, 1958; Marmorston, Crowley, Myers, Stern, and Hopkins, 1965; MacMahon *et al*, 1971). However, these observations are in part unconfirmed and not necessarily specific to cancer of the breast (Bauld, Givner, and Milne, 1957; Hellman, Fishman, Zumoff, Cassouto, and Gallagher, 1967).

There is also evidence for involvement of the pituitary in human breast cancer. The disease is rare in women with hypopituitarism (Mustacchi and Shimkin, 1957); and enhanced calciuria from bone metastases has been described following the administration of growth hormone and of prolactin (Pearson and Ray, 1959; McCalister *et al*, 1961).

No clear relationship has so far been observed between circulating hormone levels and the disease. A tendency for women with breast cancer to secrete more growth hormone in response to a glucose load has been described by Greenwood, James, Meggitt, Miller, and Taylor (1968) and Pearson, Llerena, Samaan, and Gonzales (1968), and recently Murray, Mozaffarian, and Pearson (1972) have also reported increased levels of circulating prolactin in this disease. In our experience the growth hormone response to insulin hypoglycemia was normal in women with advanced malignancy of the breast (Stewart, Benson, Roberts, Forrest, and Greenwood, 1971) and circulating prolactin, estimated either by heterologous or homologous immunoassay, has proved to be the same in normal women, women with benign disease of the breast, and those with

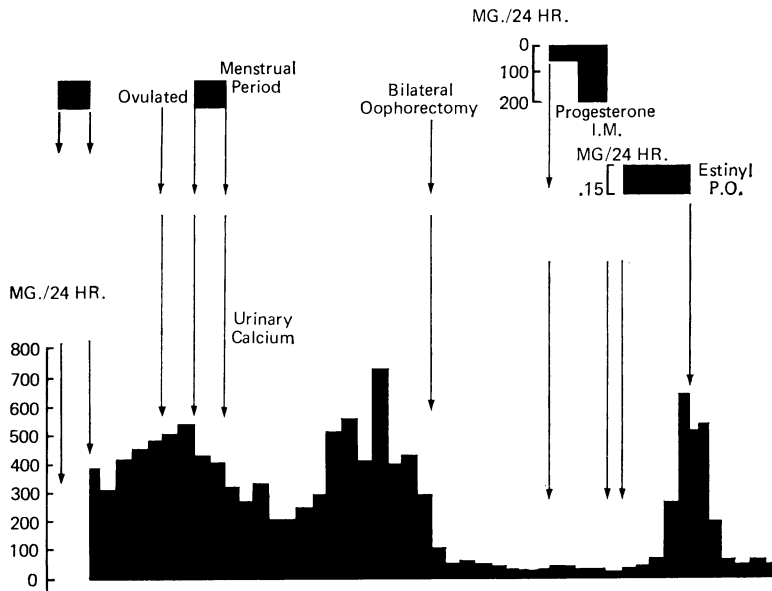


Fig 4 Urinary calcium excretion in patient with bone metastases from mammary cancer to show effect of ovarian cycle, oophorectomy, and oestrogen. (From Pearson et al, 1954.)

cancer (Forrest, 1972; Boyns, Cole, Griffiths, Roberts, Buchan, Wilson, and Forrest, 1973; Wilson *et al*, 1973). Recent results using a homologous assay are shown in the table. Nor have we found any relationship between the effect of treatment on advanced breast cancer and the levels of circulating plasma prolactin. In fact remission of disease can occur in the face of increased prolactin secretion, even when the surgical procedure is pituitary stalk section (Turkington, Underwood, and Van Wyk, 1971).

Strangely, a stronger relationship has emerged between the levels of the metabolites of the C-19 (androgenic) steroids in the urine and human breast cancer. In a series of papers between 1960 and 1971, Bulbrook and his colleagues have suggested that abnormally low levels of urinary aetiocholanolone may confer an increased risk of breast cancer, a poor prognosis of established disease, and a low incidence of response to endocrine surgery (reviewed by Forrest, 1972). Although these results are not fully confirmed by others, for example, we found abnorm-

	Number of Patients	Prolactin Level (mAmp/ml \pm SE)
Control	39	8.3 \pm 1.2
Primary breast cancer	14	10.9 \pm 2.2
Advanced breast cancer	31	8.2 \pm 0.9

Table Circulating prolactin levels in women with cancer of the breast and hospital controls

al aetiocholanolone excretion only in women with localized forms of advanced breast cancer (Cameron, Griffiths, Gleave, Stewart, Forrest, and Campbell, 1970), they draw attention to a possible role for steroid hormones other than those of ovarian origin in the disease. Aetiocholanolone is a metabolite of dehydroepiandrosterone and its sulphate secreted by the adrenal cortex. These C-19 steroids are currently of interest in view of the demonstration that human breast cancer and other breast tissues may metabolize them.

The Tumour

If human breast cancer is incubated or perfused with steroid precursors carrying a radioactive label, conversion to a range of metabolites can be demonstrated (Adams and Wong, 1968; Jones, Cameron, Griffiths, Gleave, and Forrest, 1970; Jenkins and Ash, 1972). By using different substrates one can study different steps in the steroid synthetic pathways and form a pattern such as that shown in figure 5. While there is some uncertainty regarding the biosynthesis of oestrogens (Adams and Wong, 1972; Dao, Varela, and Morreal, 1972) there is no doubt that circulating DHA-sulphate, the main C-19 steroid secreted by the adrenal, can be converted to the active androgen 5α -dihydrotestosterone. Recent observations in our laboratory indicate that similar systems also are present in fibroadenomas of the breast and in normal breast tissues (Miller,

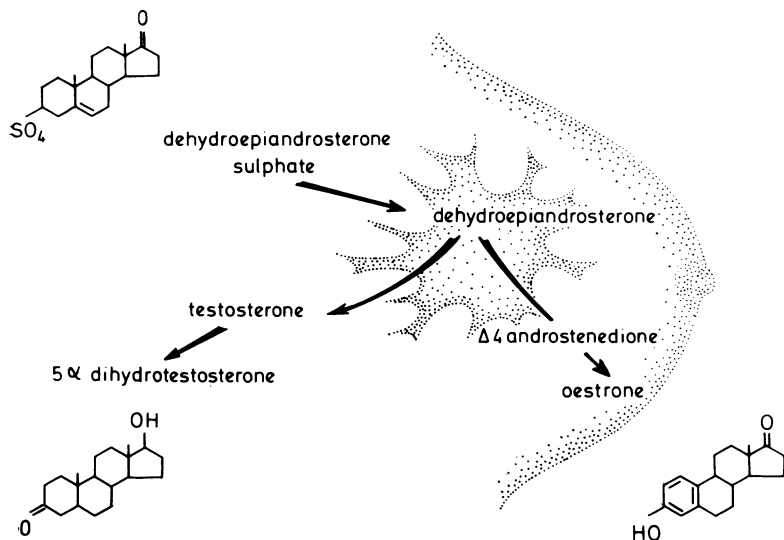


Fig 5 Steroidogenic pathways in breast cancer.

McDonald, Forrest, and Shivas, 1973). In the light of this information absolute levels of circulating hormones may be less important than the ability of the tumour to synthesize growth-promoting steroids which are locally active.

The recent work of Jensen and his colleagues from the Ben May Institute on oestrogen receptor activity has pinpointed another mechanism which may be concerned with the hormonal sensitivity of human breast cancer. Initially defined in the uterus of the immature rat (Jensen and Jacobson, 1960, 1962) it is now clear that high affinity binding protein is also present in the cytoplasm of human breast cancer (Jensen, 1970; Jensen *et al*, 1972). This cytoplasmic receptor has been identified as an 8S protein which is believed to form part of a two-step system responsible for the uptake of oestradiol by the cell and its transport to the nucleus. Of particular importance is the relationship of this oestrogen receptor to hormone dependence; it has been reported by Jensen *et al* (1972) that only one of 18 human tumours without demonstrable oestrogen receptor activity were responsive to adrenalectomy or hypophysectomy.

A further relationship recently explored is that of sulphating enzymes for oestrogens. These enzymes are responsible for the conjugation of steroid with sulphate and their identification in a tumour has also been related to their hormonal responsiveness (Dao and Libby, 1968, 1972).

It seems obvious that if we hope to unravel the role of hormones on neoplasia, more work is required on the action of hormones on the tumour

cell. What other receptor mechanisms are present? Which messenger systems are involved? How do oestrogen and other hormones influence cell growth and multiplication? These are the problems which face investigators in this field and only when they are solved can we hope to explain the phenomenon first described in 1896, yet still ill understood.

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