# Hormonal Mechanisms in Breast Cancer

## The Scientific Basis of Medicine

**Presented by Professor Patrick Forrest.**

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**Introduced by Dr Ian Gilliland**

**Directed by David Sharp.**
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**Black-and-white**
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<**Dr Ian Gilliland to camera**>

Professor Forrest is Regius Professor of Clinical Surgery in the University of Edinburgh, and Surgeon to the Royal Edinburgh Infirmary. He came to Edinburgh from the Chair of Surgery in the Welsh National School of Medicine. He is widely known for his work on breast cancer and in particular on the hormonal factors involved. The subject of this communication is hormonal mechanisms in breast cancer. Professor Forrest.

<**Professor Patrick Forrest to camera**>

In 1896, a new phenomenon was described at a meeting of the Edinburgh Medical Surgical Society. It was of remission of recurrent cancer of the breast, occurring in a

33-year-old Glasgow woman, following oophorectomy. The date of the operation was the 18th of June, 1895, the surgeon George Beatson, whose […]

<**Forrest over photograph of George Beatson, then table listing other main advances in breast cancer therapy**>

[…] photograph is shown here.

<**Table**>

| | |
|---|---|
| **Oophorectomy** | **Beatson 1896** |
| **Androgens** | **Loeser 1938** |
| | **Ulrich 1939** |
| **Oestrogens** | **Haddow, Watkinson & Paterson 1944** |
| **Adrenalectomy** | **Huggins & Bergenstal 1951** |
| **Hypophysectomy** | **Luft, Olivecrona & Sjorgren 1952** |

This was but the first of several therapeutic methods applied to advanced cancer of the breast. In 1938, we saw the introduction of androgens. In 1944, of oestrogens and subsequently, by Huggins and Bergenstal in 1951 – the performance of adrenalectomy, and then Luft, Olivecrona of hypophysectomy in the management of the advanced disease.

<**Forrest to camera**>

Interestingly, it was on hearing Olivecrona lecture on hypophysectomy when I was working in the Mayo Clinic in America that I first became interested in the hormonal mechanisms of human breast cancer.

Now, these various methods of treatment have certain things in common. Firstly, the remission rate achieved by them is remarkably similar. Secondly, one can observe a whole spectrum of remission from apparent slowing down or arrest of the disease, on the one hand, to dramatic regressions of all tumour deposits on the other. Thirdly, the

remissions achieved by these endocrine manipulations are always temporary, sooner or later the disease will become reactivated. Nevertheless, they have allowed the clear definition of human breast cancer into tumours which are endocrine-responsive, hormone-dependent tumours, and those which are not.

Now, the breasts are important attributes of all mammals. Their size and their number depend on the number of offspring in an average litter and the site of the breasts is dependent upon accessibility to the suckling infant.

Initially it was believed that oestrogen was the main hormone which controlled the growth and development of the breast but, largely as a result of the work of Folley, Reading, England and of Lyons and his colleagues in Berkeley, California, it became clear that oestrogen alone had little effect on breast development and that a whole range of hormones secreted by the pituitary, the adrenals and the ovaries were responsible for the observed changes. These studies were carried out in adrenalectomised, hypophysectomised and oophorectomised rats and it was found […]

<**Forrest over diagram showing hormones essential for duct growth, then to camera**>

[…] that even the simplest of the developments, that of duct growth, was dependent upon three hormones – oestrogens, adrenal cortisol and growth hormone. For full lobuloalveolar development, equivalent to that of late pregnancy, one required a growth tetrad of oestrogen, progesterone, prolactin and growth hormone. This hormone, prolactin, being essential for the development of assen or alveoli at all. And then in the fully developed breast for the initiation of lactation one requires the lactational triad of prolactin, growth hormone and cortisol.

Although one cannot carry out similar studies in humans, nevertheless, there is good reason to suspect that the mechanisms governing the development and growth of the human breast are no less complex.

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With experimental tumours, oestrogens again were implicated early on as the likely hormone promoting their growth. The early work of Loeb, Lacassagne, Murray, with spontaneous breast cancer in mice which, as you know, is due to the milk virus, they showed that the promotion or induction of these tumours was dependent upon oestrogens. Thus oophorectomy in female mice reduced the incidence of spontaneous breast cancer, whereas the administration of oestrogens to male mice, normally more or less immune, except for one unique strain, more or less immune from spontaneous breast cancer, did induce tumours.

Evidence that the pituitary was also implicated was not long delayed. And it was shown in the 1930s that hypophysectomy also reduced the incidence of spontaneous breast cancer in mice where the grafting of extra pituitaries increased it. And in 1958, Furth and Clifton described a particular and peculiar tumour of the pituitary which could be induced by radiation and which secreted, it was believed, […]

<**Forrest over illustration of mouse, then back to camera**>

[…] prolactin and growth hormone and which was associated with mammary hyperplasia and a high incidence of cancer. Here was clear evidence that pituitary hormones affected the incidence of cancer in mice.

Oestrogen and prolactin also are concerned with the promotion of induced breast cancers in rats. That most frequently used is the Huggins tumour induced by 7,12-dimethylbenzanthracene given in a pulse dose, either intravenously or by intragastric installation, and the peculiar property of these tumours in the rat is their hormone dependence.

<**Forrest stands and refers to series of graphs and charts on a board, then to camera**>

On this chart we see the growth pattern of a tumour induced in our laboratory, showing how following induction there is a regular growth in size of the tumour. When the ovaries were removed the tumour went into regression and then when oestrogen was administered the growth resumed. Here then is good, clear evidence that oestrogen affects the growth of the Huggins DNB-induced tumour of rats. But so also does prolactin and it was subsequently shown that hypophysectomy had similar effects to those you have seen following oophorectomy and the administration of anti-prolactin, as Pearson showed, also inhibited the growth of these tumours. Similarly, the grafting of extra pituitaries, the production of a state of hyperprolactinaemia in the rat increases the growth rate of these tumours. Therefore they are dependent both on oestrogen and on prolactin. Which is the primary hormone concerned in the promotion of those tumours one cannot say, for it is known in the rat, and has been confirmed in other species that these two hormones mutually stimulate each other. Thus the administration of oestrogen leads to a stimulation of prolactin secretion by the pituitary. Conversely, the administration of prolactin may lead to increased synthesis of oestrogens.

As far as humans are concerned, there is of course less evidence, or it's more difficult to accrue evidence, that these two hormones, or for that matter any other hormones, are directly implicated. Nevertheless there are clear guidelines that they are indeed so.

**00:10:24:00**

<**Forrest over diagram detailing the effects of oestrogen on a case of metastatic breast cancer, then to camera**>

For example, if one takes patients with bone metastases from advanced breast cancer, one can on occasion monitor behaviour of these metastases by estimating the urinary calci. This chart is taken from work by Pearson in the Memorial Centre in New York – he's now in Cleveland Ohio – showing how, in a young woman with bone metastases, the hypercalciuria cycled with the ovarian cycle. And how, following bilateral oophorectomy, the urinary calcium fell to low levels indicating healing of the

metastases, and then on administering a small amount of oestrogen by mouth to the patient, hypercalciuria was re-induced.

So, oestrogen-dependence was regarded, just as in the experimental tumours, as the likely mechanism of hormone sensitivity in human breast cancer. And then, when Woolley, and his colleagues in Chicago, showed that if you removed the ovaries of mice at birth, they would still develop normal mammary changes – hyperplasia of the breast, growth of the breasts, associated with nodular hyperplasia of the adrenals – it was reckoned that the adrenals also secreted oestrogens and that adrenalectomy was an effective procedure because of removal or deprivation of circulating oestrogens.

<**Forrest over graphs showing changes in oestrogen levels following adrenalectomy, to camera in between**>

And this concept was considerably strengthened by the observation by Dowell and Huggins that those women who responded to adrenalectomy had, in their urine, collected pre-operatively, higher amounts of oestrogen than were found in those women in whom response to adrenalectomy did not occur.

As you are aware in the management of advanced breast cancer, adrenalectomy is reserved for the post-menopausal patient, or for the pre-menopausal woman in whom the ovaries first have been removed. The advent of chemical methods of estimating oestrogens led to a great deal of work in this country and elsewhere, in the early-1950s, to try to determine whether the pattern of oestrogen excretion could be linked up with hormone dependence.

On this chart we see a series of studies carried out in our laboratories, when I worked with Sir Charles Illingworth in Glasgow, these being estimates done by Angus Simm, and they show the urinary oestrogens, estimated chemically as oestriol, oestrone and oestradiol 17β, in women undergoing oophorectomy, adrenalectomy and pituitary ablation by atrium implant for advanced cancer of the breast. And as you can see, the pre-operative urinary oestrogen levels did not differ

between those women who responded to the oophorectomy, adrenalectomy or pituitary implant and those who failed to do so. And findings such as these were confirmed by many groups.

Nevertheless, there is some evidence, recently accruing, that circulating oestrogen levels, or urinary oestrogen levels, may still be of some importance. It has been shown, for example, by McMann and his colleagues, that the ratio of excretion of oestriol to other oestrogens may be different in those women at risk from cancer of the breast. And some minor variations also have been described in women with established disease, whether these are specific or significant is not yet certain.

**00:14:37:00**

When one looks at prolactin in relation to human breast cancer, again one gets a somewhat disappointing picture. The advent of a radioimmunoassay for estimating prolactin in the circulating plasma led to the estimates of prolactin in women with breast cancer, these being compared with normal women.

<**Forrest over graphs and diagrams showing the correlation between prolactin levels and breast cancer, to camera in between**>

On this chart, we see results which Ronald Wilson, working in my department in Edinburgh, in association with Boyns, Cole and Keith Griffiths in the Tenovus Institute in Cardiff, got using an immunoassay. Here we have a group of control women, a group of women with primary breast cancer, a group of women with advanced breast cancer. And as you can see, their prolactin levels did not differ. Nor have we been able to find any variation in prolactin levels as a result of the treatment of breast cancer by endocrine means, which links in with the response to treatment.

Concrete evidence that remission of human breast cancer can occur in the face of rising prolactin levels has come from studies of patients treated with stilbestrol, which causes hyperprolactinaemia, and also from studies carried out by Turkington

showing that even pituitary ablation may be effective therapy in the presence of high prolactin levels.

<**Forrest over illustration of pituitary stalk section, then to camera**>

The operation which he studied was pituitary stalk section in which, by division of the pituitary stalk, the pituitary is separated from the hypothalamic centres. And he showed that, of 11 women with advanced breast cancer treated by this means, 5 of 8 who responded had subsequently high prolactin levels in their circulating blood. And similarly, prolactinaemia was found in 2 or 3 women who did not respond. Therefore pituitary ablation in these patients did not effect a remission of disease by reducing prolactin levels.

If we now turn to a completely different group of steroids, the C19-adrenal steroids, reactive metabolites of which are androgens, a stronger relationship has strangely emerged in relation to human breast cancer. In a series of publications between 1960 and 1971, Bulbrook, Hayward and their colleagues from the Imperial Cancer Research Laboratories, have shown that the absolute level of etiocholanolone in the urine, a C19-steroid metabolite, is related to the risk of developing breast cancer, is related to the prognosis of established disease and to the endocrine responsiveness of advanced cancer.

<**Forrest over graphs and charts detailing the relationship between C19-adrenal steroids and breast cancer, to camera in between**>

On the next chart you will see results taken from Gustave Russie Institute in Paris and reported by Paul Juret. Each of these blocks represents the sum of two androgen metabolites, C19-steroid metabolites in the urine, the sum of aetiocholanolone and androsterone. And the patients were treated all by a single method, atrium implant of the pituitary, and as you can see the percentage response in those patients with high levels of androsterone plus aetiocholanolone was considerably greater than in those in whom considerably smaller amounts were present.

Now, aetiocholanolone and androsterone, as I've said, are metabolites of the adrenal C19 series of hormones. On the next chart you will see that their presence in the urine is dependent on the secretion from the adrenal, of androstenedione and dehydroepiandrosterone sulphate. I have also put in on this chart the glucocorticoid or cortical secretion side of the pathways, resulting in the urinary excretion of 17-hydroxycorticosteroids, for initially it was the ratio of aetiocholanolone to 17-hydroxycorticosteroids which formed the basis of the discriminant function described by Bulbrook in which he was able to determine, by which he was able to determine, those patients more likely to have hormone sensitive tumours. It is now quite clear from his work, and that of others, it is aetiocholanolone and not its ratio to 17-hydrocycorticosteroids which is of importance. However, one must make it absolutely clear that aetiocholanolone is only one factor which in some way determines the response of a tumour to endocrine manipulations and that many others also are concerned.

While in Cardiff, working again with Keith Griffiths and Ewan Cameron, we performed aetiocholanolone estimates in large numbers of women, control women, women with benign breast diseases, women with primary breast cancer, women with advanced breast cancer of various types, and we described low aetiocholanolone levels only in rather a restricted group of patients, those with advanced breast cancer, which was predominantly localised. And, as you know, localised disease, this slow-growing local disease without dissemination, is particularly resistant to endocrine treatment.

**00:21:00:00**

<**Forrest to camera, then over diagram showing some aspects of the endocrine dependence of tumours, then back to camera**>

Now, how do these changes which I have described relate to the endocrine dependence of tumours? Recently, it has been shown that human cancer of the breast can function in a para-endocrine way. The work of Adams and Wong, in Sydney, Australia, of Keith Griffiths and his group in Cardiff, and now of Bill Miller,

working with me in Edinburgh, has shown quite clearly that human breast cancer can take the inactive C19 precursor of adrenal origin and can convert it, by steroid biosynthetic pathways through dehydroepiandrosterone, to testosterone and even by 5-α-reductase to the active metabolite of testosterone, 5-α-dihydrotestosterone, the essential growth-promoting hormone of the human prostrate.

Several people, these three groups, have looked with great care for production of oestrogen, for obviously, if one could show the conversion of the C19-steroid to oestrogen at tumour level, this would again raise the whole concept of oestrogen dependence being of prime importance. Only in 3 tumours described in the world literature has such demonstration of oestrogen-synthesis been produced, and that is controversial. Bill Miller has been looking very carefully at many tumours in our laboratories and has so far failed to show any clear evidence of production of oestrogen by this system. He has, however, shown that production of 5-α-dihydrotestosterone is in part dependent on prolactin, for in the Huggins experimental rat tumour, a state of hyperprolactinaemia is associated with high activity of 5-α-reductase, high production of 5-α-dihydrotestosterone, in the tumour.

Nevertheless, oestrogens still are important and the work of Jenson and his colleagues in Chicago is of particular relevance. They have demonstrated that human breast cancer, like the immature rat uterus, contains in the cytoplasm of the cells […]

<**Forrest over diagram showing the activity of a receptor protein in the cytoplasm of the tumour cell, then to camera**>

[…] an 8S protein which has the property of a high affinity for binding oestradiol. And it is believed that the presence of this receptor protein in the cytoplasm of the tumour cell is a mechanism by which one can trap the small amounts of circulating oestrogen through the tumour. And it is also believed that this forms part of a 2-step mechanism by which active oestrogen, attached to the protein, can be taken into the nucleus.

What is of particular relevance and interest is the association of the presence of high-affinity oestrogen binders and hormone dependence. It's now been shown by several groups that high-affinity oestrogen-binding protein is present in about 50% of primary breast cancer cells. And secondly, shown initially by Jensen and confirmed now by some others, that the presence of oestrogen receptors in the cytoplasm of human breast cancer is associated with a higher degree of hormone dependence and very few patients in the literature, who have tumours without this protein present, have responded to adrenalectomy, hypophysectomy or other means of endocrine manipulation. So here is one possible way by which one might predict the hormone responsiveness of a tumour.

<**Forrest to camera, then over illustration showing a tissue culture experiment, then back to camera**>

The third point of interest, when studying the tumour, is the use of methods of organ culture, as shown in the simple diagram here. The principle behind the method is the culture of small fragments of tumour on grafts in culture media. By adding hormones to the culture medium one can study their effect on the morphology of the tumour on the graft, and one can also look at certain specific functions such as DNA synthesis or for evidence of growth. And this system is being used now in an attempt to predict hormone sensitivity in vitro.

As far as I'm aware, the first demonstration of the use of this type of in vitro culture was by Olga Meodusca[?] who came from Warsaw and who studied the morphology of breast tumours grown in organ culture in this way, in the presence of various hormones. And, more recently, Sally Hobbs and their group in the Westminster have been using a dehydrogenase stain to detect activity of a metabolic nature in these small fragments of tumour as a result of adding hormones to the culture fluid. Here one may well find a method for detecting not only hormone responsiveness in tumours but for studying in greater depth the effect of hormones on fundamental cell processes.

It is obviously not yet clear why the patient described by Beatson had a remission of her breast cancer. Nevertheless it is clear that hormonal influences are important determinants of the behaviour of human cancer of the breast. Further, it has recently been shown by McMann and his colleagues that the risk of developing cancer of the breast is materially altered by events of great endocrine magnitude in the early years of the human female – for example, the age at first pregnancy or early removal of the ovaries are important determinants of breast cancer risk. The discovery of intrinsic mechanisms in the tumours for binding and for conversion of steroids, and of methods to determine the effects of these hormones in vitro, are obvious important advances, and these already are proving to be more rewarding than the study of circulating hormone levels which have been conducted in the past. And they offer, for the first time, methods by which the hormone responsiveness of a tumour might be predicted.

The practical implications of this are great for it will lead to the more rational use of endocrine manipulations, not only in the patient with advanced breast cancer but in the patient with early disease. And as a result one may be able to reduce the staggering mortality of this condition in the human female. Then can the full implications of Beatson's original phenomenon be realised.

<**End credits**>