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Effects of Oestrogens on Cell Proliferation and Cell Cycle Kinetics. A Hypothesis on the Cell Cycle Effects of Antioestrogens

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INTRODUCTION

IT HAS long been known that oestrogens can provoke extensive cell proliferation in many of their target tissues, especially those in the reproductive tracts of immature and ovariectomized animals [1–3]. Oestrogens also induce cell proliferation in some neoplastic tissues. Beatson's original description of human mammary carcinoma which regressed after oophorectomy suggested a growth-promoting role for ovarian factors in some human breast cancers [4]. The subsequent demonstration that administration of small doses of oestrogens could reactivate the growth of tumours that had regressed following oophorectomy confirmed such a role for oestrogens [5]. This class of steroid hormones has also been implicated in the regulation of growth and metabolism of a number of experimental animal tumours including dimethylbenzanthracene (DMBA)- and *N*-nitrosomethylurea (NMU)-induced rat mammary carcinomas [6, 7], R3230AC and MT.W9A transplantable rat mammary adenocarcinomas [8, 9], TPDMT-4 and MXT-3590 mouse mammary tumours [10–12], carcinogen-induced rat endometrial cancer [13], rat anterior pituitary tumours [14, 15], rat prostatic cancer [16, 17] and renal carcinoma in the hamster [18, 19]. Detailed studies employing these model systems have greatly enhanced our understanding of the hormonal control of tumour growth.

The effects of oestrogens on tumour growth and tumour cell proliferation may be both direct and indirect. Direct effects are most easily seen *in vitro* and there is now appreciable evidence for direct oestrogenic stimulation of cell proliferation in both normal [20–25] and neoplastic [26–28] cultured target cells. There is also strong evidence that oestrogens have indirect effects on tumour cell proliferation which are mediated by other hormones and growth factors. For example, it appears that most of the oestrogenic effects on DMBA-induced rat mammary tumours are mediated by oestrogen-induced changes in prolactin synthesis and release [29]. In other studies Sirbasku and Benson [30, 31] have demonstrated that three cell lines which are unresponsive to oestrogen *in vitro* show oestrogen-responsiveness when transplanted into animals. These authors propose that the mitogenic effects of oestrogen *in vivo* are mediated by oestrogen-induced growth factors (oestromedins) in a manner analogous to the regulation of body growth via the induction of the polypeptide somatomedins by growth hormone.

Since oestrogens are known to influence the growth of some human mammary carcinomas a number of synthetic oestrogen antagonists have been studied as potential therapeutic agents for the treatment of this disease. Objective remissions in advanced breast cancer have been reported with the synthetic nonsteroidal antioestrogens clomiphene [32], nafoxidine [33], tamoxifen [34–36] and trioxifene [37]. Tamoxifen is now in widespread clinical use but the molecular basis of its antitumour activity has yet to be clearly defined. Both direct and indirect effects on hormone-responsive tumour cells have been postulated [38, 39].

In recent years tamoxifen has been incorporated into therapeutic regimens containing cycle- and phase-specific cytotoxic agents [40–42]. It is our belief that a more rational planning of such therapy may well be aided by an understanding of the effects of synthetic nonsteroidal antioestrogens on cell cycle kinetics, but unfortunately there has been a paucity of published data on this topic.

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In contrast, a considerable amount is known about the mechanisms by which oestrogens control cell proliferation and cell cycle kinetics. This may provide useful clues as to the loci of antioestrogen action if the current belief that antioestrogens exert their antitumour activity by virtue of their antioestrogenic properties alone is correct.

In this article, therefore, the known effects of oestrogens on cell proliferation are reviewed and their loci of action within the cell cycle identified. These data allow the formulation of an hypothesis as to the potential cell cycle effects of nonsteroidal antioestrogens. This should facilitate the design of experiments aimed at elucidating the cycle- and phase-specific effects of antioestrogens on human tumour cells and perhaps the more efficacious use of antioestrogens in combination chemotherapy.

THE CELL CYCLE

The concept of a cell cycle has been introduced to define various stages of interphase (Fig. 1) between the completion of mitosis in a cell and the completion of the subsequent mitosis in its daughter cells. G_1 phase is defined as the period between the end of mitosis and the subsequent onset of DNA synthesis. This is the phase which varies most in length so the duration of G_1 is an important determinant of the rate at which a given cell population proliferates [43–45]. This variability has been explained by two main models, referred to as the deterministic [44] and probabilistic [43, 45] models. S phase is the period during which DNA replication occurs, while G_2 phase is the period between the completion of DNA synthesis and the onset of mitosis (M).

Not all cells are actively progressing through the cell cycle, indeed most animal cells *in vivo* are not proliferating. These non-cycling cells have been regarded by some investigators as being in a separate phase, termed G_0, which is qualitatively distinct from G_1 [46, 47], whereas others have postulated that they are merely retained in G_1 for a prolonged period [45]. There may, in fact, be more than one state of quiescence since, for example, 3T3 cells deprived of serum were arrested at a stage which resembled early G_1 on the basis of cellular RNA content, whereas the same cells grown to confluence appeared to enter a deeply quiescent state with a marked loss of cellular RNA below that seen in early G_1 phase [48].

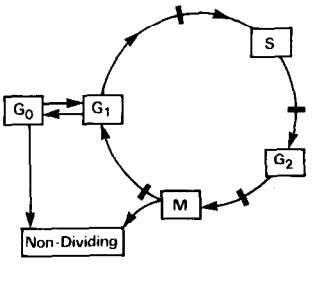

Fig. 1. A schematic representation of the cell cycle. G_1 and G_2 are the intervals between the DNA-synthetic phase (S) and mitosis (M). G_0 is a hypothetical resting state from which cells may be stimulated to re-enter the cell cycle.

Cell populations in both normal and neoplastic tissues are continuously turning over, i.e. cells are continuously being produced by mitosis and lost by cell death. The relative magnitude of these two processes will determine whether the cell population increases, remains static or declines. Any factor which changes the growth fraction, i.e. the percentage of cycling cells, the length of any phase of the cell cycle or the rate of cell death, would influence the fate of a particular cell population. Oestrogens and antioestrogens could exert their primary influence on tumour growth at any or all of these points.

EFFECTS OF OESTROGENS ON CELL PROLIFERATION AND CELL CYCLE KINETICS

Oestrogens have complex and apparently contradictory effects on cell growth. At low doses they often act as mitogenic agents and induce cell proliferation, while at high pharmacological doses they may inhibit cell proliferation [9, 26–28, 49, 50]. This biphasic dose–response curve may explain why high-dose oestrogens are sometimes effective as antineoplastic agents [51, 52].

The earliest studies on the effects of oestrogens on cell proliferation were performed *in vivo* and for this reason it was not possible to distinguish between direct and indirect effects of the hormone. Bresciani's studies [53, 54] comparing the alveolar cells of mammary glands from intact mice with the same cells from animals which were ovariectomized and treated for 3–4 days with 1 μg oestradiol and 1 mg progesterone showed that hormone treatment decreased the time that these cells spent synthesizing new DNA, i.e. decreased the length of S phase. A similar effect of oestrone on the duration of S phase in mouse uterine luminal epithelium was subsequently reported by Epifanova [55]. In these studies adult ovariectomized mice were given two subcutaneous injections of 2.5 μg oestrone at 24-hr intervals followed 24 hr later by an injection of tritiated thymidine. Animals were killed at various times after thymidine injection and the cell cycle kinetic parameters calculated from labelled mitoses curves. A 1.5-fold reduction in the generation time was noted and this was due to a shortening of both the G_1 and S phases [55]. In the same study animals were given repeated injec-

tions of tritiated thymidine to determine the percentage of cycling cells, i.e. the growth fraction. Oestrone treatment increased the growth fraction greater than 3-fold in the luminal epithelium and greater than 2-fold in the glandular epithelium. Another interesting observation by this author was that the continued administration of oestrone (six or more daily injections) led to a decrease in the mitotic index with a new increase in the number of mitoses on withdrawal of the hormone. Autoradiographic analysis showed that depression of mitotic activity was apparently due to a block in the ability of the cells to proceed through G_1 into S phase, with the subsequent onset of DNA synthesis in a large number of cells when hormone levels fell and the putative block was removed [56].

Numerous other studies have investigated the effects of oestrogen on cell proliferation in the genital tracts of ovariectomized mice and rats [57–80]. While there were some discrepancies among the early studies (reviewed by Das [69]), it is now generally agreed that oestrogens control the proliferation of luminal and glandular epithelial cells in the ovariectomized mouse in the following ways. Oestrogens can induce the semi-synchronous recruitment of a population of quiescent or G_0 cells into the cell cycle, thus increasing the growth fraction [54, 55, 67, 71, 72] while at the same time reducing the generation time of cycling cells by shortening both G_1 [55, 60, 61, 69, 71, 72] and S phases [53–55, 60–62, 65, 68, 69, 71, 72] and perhaps $G_2 + M$ [60, 66, 69, 72].

An explanation for the oestrogen-induced shortening of S phase has been provided by Leroy *et al.* [79]. It is known that eukaryotic DNA molecules consist of many replicative units (replicons) which are arranged end-to-end. DNA synthesis is initiated at the middle of each replicon and proceeds bidirectionally at divergent fork growing points until fusion occurs between adjacent sections. Assuming that an increase in DNA synthetic rate could be due to an increased rate of replication along pre-existing replicons, an increase in the number of simultaneously operating replicons or both, Leroy *et al.* [79] demonstrated that the oestrogen-induced increase in the rate of DNA synthesis in ovariectomized mouse uterine epithelium following oestrogen treatment was due to an increase in the rate at which individual replicons were replicated. The 50% increase in fork growing speed seen in this study would account for a 33% reduction in the duration of S phase [79].

There are a number of other interesting observations from these studies *in vivo* which may be important in understanding the mechanisms by which oestrogens control cell proliferation. Martin *et al.* [71] demonstrated that the ability of rat uterine luminal epithelium to respond to a second injection of oestradiol was inversely related to the proportion of cells remaining in the DNA synthetic phase or mitosis. They suggested that cells in these phases of the cell cycle were essentially insensitive to the stimulatory effects of oestradiol on growth. Other experiments in which animals were continuously exposed to oestrogen demonstrated distinct waves of proliferative activity and cell death in the uterine epithelium [70]. The author suggested that the decrease in proliferative rate may have been due to the increased cellular density, with another wave of DNA synthesis and mitosis following a period of increased cell death [70]. Under the same conditions of oestrogen stimulation, mitosis in the vaginal epithelium did not show the same fluctuations [80]. The constraints of cell density probably do not apply in the vagina, since in this organ cells migrate out from the basal layer, become keratinized and are shed. Thus the proliferative response to the same hormonal regimen was tissue-specific. Further evidence for this was seen in the experiments of Eide [72], where a dose of oestrogen which induced cell proliferation in the body of the uterus had exactly the opposite effect on the cervical epithelium, i.e. it decreased the proliferation rate by decreasing the growth fraction and increasing the cell cycle time by prolonging both G_1 and S phases [72].

Even within the uterine epithelium itself, Das [69] noted that the glandular cells consistently had higher grain counts than luminal cells responding to oestrogen, despite the fact that the rates of DNA synthesis were essentially the same in both tissues. This result was interpreted as indicating greater and more rapid availability of tritiated thymidine in the gland cells, but may well indicate fundamental differences in nucleotide biochemistry in the two different cell types which may ultimately be found to be important in their differential responses to oestrogen and antioestrogen.

There are few data on the effects of oestrogens on cell cycle kinetics of mammary carcinoma cells *in vivo*. The only major published study related to the effect of hormone withdrawal on the cell proliferation kinetics of GR mouse mammary tumours induced by oestrone–progesterone treatment [23]. Experimental data consisted of growth curves, percentage-labelled-mitoses curves and labelling indices. When these data were fitted to mathematical models by computer-assisted methods they were interpreted as showing that hormone deprivation was accompanied by a decrease in growth fraction and a decrease in cell

production rate due to an increase in the mean cycle transit time, particularly in the G_1 phase. The rate of cell loss was unchanged. Presumably hormone administration had the opposite effects, i.e. it increased cell production rate by increasing the growth fraction and decreasing the cycle time [23].

The first evidence that oestrogenic effects similar to those seen *in vivo* could be reproduced *in vitro* came from studies on primary cultures of rabbit endometrium grown in chemically defined medium [24]. These cultures were found to be a mixture of cycling (20%) and non-cycling or G_0 (80%) cells. Addition of 10^{-7}M diethylstilboestrol to the medium increased the proportion of proliferating cells to 80% and significantly shortened the generation time by decreasing the length of both G_1 and S phases [24]. In a subsequent study, where cycling and non-cycling cells were separated, it was demonstrated that only the quiescent cells were targets for the growth effects of oestradiol [81].

Oestrogens have also been shown to stimulate cell proliferation in two mammary carcinoma cell lines, MCF 7 [26, 27] and ZR 75-1 [28], although data with the former cell line vary with laboratory [26, 27, 82–84], possibly due to the existence of different clones of MCF 7 cells or to different culture conditions. Cell cycle kinetic data were available from only one study [27], where 10^{-9}M oestradiol stimulated cell proliferation and 10^{-7}M inhibited growth. The lower dose was shown to shorten the overall cell cycle time, and since it also increased the proportion of cells in S phase it was considered that the predominant effect was a reduction in the duration of the G_1 phase. At 10^{-7}M, oestradiol caused a decrease in the proportion of S phase cells compared to controls, which may have been accounted for by either a relative prolongation of the other phases of the cell cycle, a reduction in the proportion of cycling cells or both [27].

At even higher concentrations, i.e. 10^{-6}–10^{-5}M, oestradiol inhibited the incorporation of tritiated thymidine into DNA and eventually killed both oestrogen receptor-positive and oestrogen receptor-negative human mammary carcinoma cells [26, 85]. This effect appears to be mediated independently of the oestrogen receptor since it is unaffected by inhibitors of RNA and protein synthesis [85] and can be induced, at similar concentrations, by ligands which have very weak affinity for the oestrogen receptor, e.g. 17α-oestradiol [26]. Nonspecific effects of hydrophobic steroids on the cell membrane and cytoskeleton structure have been suggested as possible primary steps in the inhibition of cell growth by high-dose oestrogens *in vitro* [85]. Another interesting effect of high-dose (2×10^{-5}M) oestradiol was to induce a mitotic delay in HeLa cells [86]. Only those cells that were in S or G_2 periods at the time of oestradiol treatment were affected. It was suggested that these effects were mediated by the negative charge of the oestradiol molecule since the effects could be reversed by the cationic molecules calcium chloride and putrescine [86].

There are few data on the effects of oestrogens on cell death rate. Martin noted that in mouse uterine luminal epithelium, cell death was minimal following treatment with oestradiol. However, if oestrogen treatment was not continued, mitotic rates fell, cell death increased and cell numbers declined [75]. Similar studies with the short-acting oestrogen oestriol demonstrated that this compound stimulated luminal cells to enter DNA synthesis and mitosis, but cells died prematurely and cell numbers did not increase [73]. This suggests a continuous requirement for oestrogen in order that cells complete the cell cycle. Some recent data from studies *in vitro* can be interpreted as support for but not proof of the concept that oestrogen deprivation increases the rate of cell death. When ZR 75-1 cells growing in defined medium were deprived of oestradiol, cell numbers became static, yet cells continued to incorporate tritiated thymidine, albeit at a lower rate than hormone-treated cells. When the cells were prelabelled with thymidine, 60% of the label was lost into the medium of oestrogen-deprived cells compared with 1% loss in oestrogen-treated cells [87]. Thus it appears likely that oestrogen deprivation not only decreases the birth rate of cells through lengthening of the cell cycle and perhaps the production of non-cycling G_0 cells but may also increase the rate of cell death. Both processes may thus contribute to regression of hormone-dependent tumours following hormone deprivation.

EFFECTS OF ANTIOESTROGENS ON CELL PROLIFERATION AND CELL CYCLE KINETICS

There is very little published information on the effects of antioestrogens on cell proliferation and cell cycle kinetics. As with oestrogens, the majority of data is derived from experiments using the reproductive organs of immature and castrated rats and mice. Interpretation of these data is complicated by large species differences in the response to antioestrogens.

In the mouse uterus and vagina, nonsteroidal antioestrogens are oestrogenic [88]. Whether this is due to the production of oestrogenic metabolites of antioestrogens or to inherent properties of mouse target cells remains to be elucidated. In the

uterus they reduce uterine luminal cell death, induce as much epithelial proliferation as oestradiol and are incapable of inhibiting oestradiol-induced cell proliferation [74, 75]. However, pretreatment with tamoxifen did produce refractoriness to oestradiol in the mouse vaginal smear test [89, 90]. Interestingly, antioestrogens nevertheless still have antitumour activity in mice, as evidenced by their ability to inhibit mouse mammary tumour growth in GR mice [12] and human MCF 7 tumours grown in nude mice [91].

In the rat tamoxifen always stimulates vaginal proliferation, though not as much as oestrogen. Mitotic indices are also increased, being higher than those induced by oestradiol at early times but lower at later time points [75]. The situation is more complex in the rat uterus since this organ contains several classes of functionally different cell populations, e.g. luminal epithelium, glandular epithelium and stroma, which respond differently both to steroid hormones and to antioestrogens. Kang *et al.* [92] were the first to demonstrate that an antioestrogen, CI 628, induced cell proliferation in the uterine stroma but suppressed it in the epithelium. It was subsequently confirmed that rat uterine epithelial cells undergo prolonged hypertrophy but not hyperplasia following antioestrogen treatment [74, 75, 78, 93]. More recent detailed studies by Martin [74, 75] revealed that tamoxifen induced more stromal labelling with tritiated thymidine than did oestradiol, but it was unclear how many cells that incorporated label went on to divide since mitotic cells were rare. In the luminal cell population tamoxifen induced prolonged hypertrophy with only small increases in labelling index and cell numbers. Mitotic index and cell death rates did not increase significantly. Tamoxifen thus appeared to be a pure antagonist for luminal cell proliferation since it failed to induce a proliferative response of its own and completely inhibited the increase in cell number induced by continuous oestradiol treatment [74, 75]. Similar findings have been reported with nafoxidine [94]. In the glandular epithelium antioestrogens appeared to be cytotoxic. Tamoxifen initially induced hypertrophy with a small transient increase in labelling index. However, it also increased cell death rate and this process was not reversed by oestradiol. The net result was a substantial reduction in gland cell numbers [74, 75]. These differential cellular responses, which are unlikely to be explained by differences in tamoxifen metabolism [95], may have important implications for understanding the cellular selectivity of the antiproliferative effects of nonsteroidal antioestrogens.

The extensive literature on the effects of antioestrogens on tumour growth *in vivo* [96, 97] has, to date, provided little insight into the cell-cycle effects of these drugs. While it is clear that antioestrogens can inhibit the growth rate and induce regression of many oestrogen receptor-positive tumours [96, 97], it is not known whether this is due to a decrease in cell birth rate, an increase in death rate or both. The general consensus of opinion at present seems to be that the more important effect is inhibition of oestrogen-stimulated cell proliferation, although some recent *in vitro* data discussed below suggest that the question remains open.

Interestingly, there have now been several reports of antioestrogens stimulating mammary tumour growth both in experimental animals [11, 98] and humans [99, 100]. Whether this is a direct effect on tumour cells or is mediated by antioestrogen stimulation of growth factors which induce cell proliferation remains to be elucidated.

More detailed information on the effects of antioestrogens on cell proliferation comes from studies *in vitro* with human mammary carcinoma cell lines. In two oestrogen receptor-positive lines, MCF 7 and ZR 75-1, antioestrogens have been shown to cause growth inhibition and to decrease incorporation of tritiated thymidine into DNA [26, 28, 82, 84, 101]. These effects were reversed by the simultaneous or subsequent administration of oestradiol, which is compatible with the effects of antioestrogens, being oestrogen receptor-mediated [26, 102]. It is of interest that the rate of tritiated thymidine incorporation into cells receiving oestradiol 24–36 hr after tamoxifen treatment was significantly greater than in cells treated with oestradiol alone [26]. A probable explanation for this observation is that tamoxifen blocks cells at a point in the cell cycle in such a way that when they are released from this block by the addition of oestradiol, a synchronous cohort of cells moves into S phase.

In the light of some recent findings, however, there remains some doubt as to whether all the antiproliferative effects of antioestrogens are mediated by the oestrogen receptor. For example, in chemically-defined serum-free medium, tamoxifen inhibited growth of ZR 75-1 cells to a greater extent than removal of oestradiol from the medium [28]. Similar observations with MCF 7 cells were alluded to in an earlier study [103]. While these data could possibly be explained by antagonism of the effects of residual oestrogen, this now seems unlikely [87, 104], and a more plausible explanation is an effect of tamoxifen which is not influenced by oestrogen [28, 87, 105]. Support for this latter concept comes from the

observation that prolonged exposure to tamoxifen (i.e. greater than 60 hr) cannot be reversed by oestradiol [87].

DISCUSSION

This brief review, which summarizes the published data concerning the effects of oestrogens and antioestrogens on cell proliferation and cell cycle kinetics, highlights the lack of detailed understanding of the effects of nonsteroidal antioestrogens on these parameters. As stated in the introduction, we believe a more complete understanding of these phenomena would not only enhance our understanding of the molecular basis of the antitumour activity of nonsteroidal antioestrogens, but may also facilitate a more rational incorporation of these compounds into combination chemotherapeutic regimens. Since it is generally believed that antioestrogens are antitumour agents because of their antioestrogenic properties, one can use the somewhat more detailed data on the cell cycle effects of oestrogens to formulate an hypothesis as to the likely sites of action of antioestrogens within the cell cycle since the loci at which oestrogens and antioestrogens act would be expected to coincide.

Studies on the cell cycle effects of oestrogens have been conducted mainly *in vivo*, but documentation of similar responses *in vitro* suggests that many effects are due to the direct action of oestrogens on their target cells. Physiological and low pharmacological doses of oestrogen can induce cell proliferation in many oestrogen target cells and this appears to be due to: (1) an increase in the size of the proliferating cellular pool due to recruitment of non-cycling cells into the cell cycle, i.e. increasing the growth fraction; (2) a shortening of the overall cell cycle time due chiefly to a reduction in the length of G_1 phase and, to a lesser extent, of S phase, and possibly also of $G_2 + M$; and (3) a decrease in cell death rate. While this is obviously an oversimplification and does not take into account differential tissue responses to oestrogen or cell density constraints on continuous proliferation, these three major changes in cellular kinetics appear to apply to most cell populations whose growth rates are stimulated by oestrogen. If these are the major cell cycle effects of oestrogens, and assuming the antitumour effects of non-steroidal antioestrogens are due to their antioestrogenic properties, we hypothesize that nonsteroidal antioestrogens would: (1) decrease the growth fraction, i.e. increase the proportion of G_0 cells; (2) increase the cell cycle transit time by a lengthening of G_1 and, to a lesser extent, S phase; and (3) increase the rate of cell death.

Experimental evidence to support this hypothesis is, to date, very limited. There are no data to support the idea that antioestrogens decrease the growth fraction—it appears that it has not been tested. Recent flow cytometry data from this laboratory indicate that antioestrogen-treated cells accumulate in the G_0/G_1 phase of the cell cycle [105]. Whether this is due to the production of G_0 cells, a lengthening of G_1 relative to the other phases of the cell cycle or to arrest at a specific point in G_1 remains to be elucidated. Certainly, these data in conjunction with tritiated thymidine incorporation studies [26] indicate that fewer cells are synthesizing DNA, i.e. are in S phase, following antioestrogen treatment. Similarly, the fact that tamoxifen-treated cells move into S phase in a semisynchronous manner following 'rescue' with oestradiol [26] could be interpreted as evidence for either production of G_0 cells or arrest in G_1. There are few data on the effects of antioestrogens on cell death. Tamoxifen appeared not to influence cell death rates in luminal epithelium of rat uterus, but markedly increased death rates in the glandular epithelium [74, 75]. This again illustrates the complexity of the mechanisms of action of this drug and its differential tissue responsiveness.

In view of the differential species and tissue responses to antioestrogens, it may not be valid to extrapolate from studies in rodent reproductive tissues to human mammary carcinoma *in vivo*, but despite the dearth of knowledge, working hypotheses must be based on some rational scientific data. At present, all that can be said about the effects of antioestrogens on cell proliferation and cell cycle kinetics of human mammary carcinoma cells *in vivo* is that under some circumstances these drugs can induce tumour regression by kinetic mechanisms yet to be defined. The specificity for oestrogen receptor-positive tumours *in vivo* is not necessarily paralleled *in vitro*, where receptor-negative cell lines, although less sensitive to antioestrogens, nevertheless can be inhibited in their growth with changes in the cell cycle kinetic parameters similar to those seen with receptor-positive cells (Sutherland *et al.*, unpublished observations). Whether this is an argument for antitumour activity independent of oestrogen receptor mechanisms or for mechanisms that occur *in vitro* but are absent *in vivo* remains to be elucidated.

As for the potential mechanisms by which tumour regression is achieved, there are again few data. We have postulated what one might expect to find if the drugs acted purely as antioestrogens, and there is preliminary evidence to support some of these ideas. Whether a decrease in cell birth rate is enough to explain the rate of tumour regression

seen *in vivo* or whether one has also to invoke changes in death rates is unclear. Perhaps recent data indicating that antioestrogen treatment is superior to oestrogen withdrawal [28, 87, 91] in producing tumour regression could be seen as evidence implicating antioestrogen-induced increases in tumour cell death rates.

It is undoubtedly naïve to assume that a single mechanism is responsible for all antioestrogen-induced regressions of human tumours. In addition to the direct effects of antioestrogens on tumour cells *in vitro*, one must consider the overall effects of the drug on the whole organism, e.g. its pharmacokinetics and metabolism and effects on the overall hormonal environment, which can only be fully assessed *in vivo*. In this laboratory we have opted initially for an approach at the *in vitro* level in order to define potential mechanisms which can then be investigated with appropriate *in vivo* model systems, e.g. human mammary tumours and cell lines grown in nude mice. Some of the more important questions that can be addressed using this approach appear to be:

1. What effects do antioestrogens have on the lengths of the cell cycle phases?
2. What are the effects of antioestrogens on the growth fraction of oestrogen target tissues and hormone-dependent tumours?
3. At what point(s) in the cell cycle are antioestrogens able to initiate their effects?
4. What effects do antioestrogens have on cellular death rate? Are these effects mediated via the oestrogen receptor and/or other mechanisms?
5. Are the actions of antioestrogens due wholly to oestrogen deprivation or are other factors involved?
6. What are the differences in the response of oestrogen receptor-positive and oestrogen receptor-negative tumours to antioestrogens?
7. Does drug metabolism *in vivo* modulate the response of target tissues to antioestrogens?
8. Are there intrinsic differences in the characteristics of various target cells which determine their response to antioestrogens, e.g. are there inherent differences in this regard between murine and, say, human cells?
9. What are the cell cycle effects of high-dose oestrogens? Are these or other factors the prime determinants of the tumour regression sometimes seen with high-dose oestrogens?
10. Are there negative feedback systems other than constraints of space that result in reduced cell proliferation following continuous administration of oestrogen in some normal tissues? Are the same mechanisms operative in neoplastic tissues?
11. Is there a continuous requirement for oestrogen in order that oestrogen-responsive cells complete the cell cycle or is oestrogen merely required early in the cycle to activate some initial steps in the stimulation of cell proliferation?
12. Does oestrogen exert some of its effects on human mammary carcinoma by stimulating other growth factors?
13. Can antioestrogen have direct stimulatory effects on tumour cells *in vitro*? And if so, what are the mechanisms involved?
14. If cells are 'rescued' from the effects of antioestrogens by oestradiol, do the cells progress through the cycle in a synchronous manner? Could this be used to therapeutic advantage?
15. Are antioestrogens antitumour agents because they are oestrogens or antioestrogens or is some other property of the molecule responsible for their antitumour properties?

There are currently available ample authentic human mammary carcinoma cell lines and cell biological techniques to test many of these questions which appear relevant to understanding the effects of oestrogens and antioestrogens on the cell cycle kinetics of human tumour cells. We are presently pursuing studies of this nature. It is hoped that this article will stimulate others to apply their particular expertise to finding the answers to some of these interesting and important questions.

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