Critical Components of Testicular Function and Sensitivity to Disruption

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ABSTRACT

Toxic agents can interfere with the male reproductive system at many targets. Radiation and cancer chemotherapeutic drugs represent one class of toxins the sterilizing effects of which can be analyzed qualitatively and quantitatively in terms of testicular cell kinetics. The cells most sensitive to killing by these agents are the rapidly dividing, differentiating spermatogonia. Cells past the DNA-synthetic stages, including spermatocytes, spermatids, and nongerminall cells, are generally resistant. The slow cycling stem spermatogonia show an intermediate sensitivity, but appear to be the critical targets for the resulting long-term oligo- or azoosperma and infertility. The extent of recovery of spermatogenesis and the duration of infertility can be predicted on the basis of stem cell survival alone, independent of the antineoplastic agent used. When murine stem cells are killed, regeneration of their number and repopulation of the seminiferous epithelium begin almost immediately. In man, recovery can be delayed for years after exposure to agents that kill stem cells. This is a result of the regulation of stem cell regeneration and differentiation in man, the mechanisms of which are unknown. This regulation can explain quantitative differences in interspecies sensitivities to toxic agents. For example, man is much more sensitive than the mouse to reduction in sperm count by radiation at short times after exposure, but not when sufficient recovery times are allowed.

INTRODUCTION

Humans are exposed to potential and known reproductive toxins in the workplace, the environment, foods, and cosmetics and during medical treatment. The function of the male reproductive system may often be the most sensitive to toxic effects. Indeed, azoospernia, in the absence of any other major systemic toxicities, has been documented in workers handling the pesticide dibromochloropropane (Wharton et al., 1977). Furthermore, roughly half of all male patients treated with chemotherapy for cancer and most of those treated with a regimen including nitrogen mustard and procarbazine for Hodgkin’s disease (Schilsky et al., 1980) are permanently azoospermic. With the increasing number of new chemicals and drugs in our environment, it is important to identify the ones that are potential reproductive toxins to human males prior to actual exposure. There is a need for reproductive biologists to apply their knowledge of the biochemistry and physiology of the normal processes involved in reproduction to contribute to the qualitative and quantitative determination of reproductive toxicities.

Qualitatively, it is important to identify which targets in the reproductive system are affected by the toxin. Knowledge of such mechanisms of action would then enable us to better measure these effects and to predict the reproductive toxicity of related compounds. Quantitatively, it is important to determine the doses that are toxic to the reproductive system and how various levels of toxicity affect the ultimate end point, fertility. Since testing of reproductive toxins is done in experimental animals, methods are needed for extrapolation of the dose-effect relationships seen in animals to predict doses that would adversely affect human fertility (Meistrich, 1984a).

The fertility potential of the male requires the coordinate operation of many cellular and organ systems; it can be adversely affected at many sites. The causes may be categorized as reduced sperm production by the testis, defective sperm transport and delivery, and poor quality of sperm (Table 1). Within each category many cellular or tissue targets can be affected to produce that defect; examples of adverse action of toxic agents on most of these targets have been reported.

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of stem cell renewal (Huckins and Oakberg, 1978a), the first apparent commitment to differentiation occurs when some of the Ap spermatogonia divide about two more times to produce a chain of aligned spermatogonia (Aa) that, after an extended G1 phase, differentiate into A1 spermatogonia. The spermatogonia then pass through six more mitotic cycles. Each of the cell cycles of the Ap through B spermatogonia involves a DNA-synthetic phase and a mitotic division and together these cycles constitute the main proliferative phase of spermatogenesis. The final mitotic division results in the formation of preleptotene (P1) primary spermatocytes, which perform the last round of DNA replication that occurs during spermatogenesis. Spermatocytes then pass through the stages of the meiotic prophase, after which they undergo the meiotic divisions to produce secondary spermatocytes and then haploid spermatids. Subsequently, spermatids undergo an intricate and complex process of morphologic differentiation to produce spermatozoa; there is no cell division during spermatid development.

Once the cells are committed to differentiate, the times for one cell stage to transform into the next are rigidly fixed and are related to the duration of the “cycle of the seminiferous epithelium” (Clermont, 1972) for that species. The durations of the cell stages in the mouse are indicated in Fig. 1. These kinetics of differentiation have been shown to be unaltered by exposure to various reproductive toxins or by endocrine alterations (Edwards and Sirlin, 1958; Nebel et al., 1960; Partington et al., 1964; Clermont and Harvey, 1965; Schleiermacher, 1970); the only two exceptions appear to be continuous heat (Meistrich et al., 1973) and procarbazine, which alters the kinetics of round spermatid development only (Russell et al., 1983).

The kinetics of the stem cell is less rigidly regulated and must change in response to cytotoxic insults (van Keulen and de Rooij, 1975; Huckins and Oakberg, 1978b). In the normal testis, maintenance of spermatogenesis requires that each stem cell division, on average, produce one cell that will go on to initiate differentiation and another that will remain as a stem cell. However, after stem cell killing occurs, it is apparent that the survivors must, in order to regenerate their number, pass through some divisions in which one stem cell gives rise to two. In the testis recovering from damage two processes occur: 1) regeneration,
FIG. 1. Sequence, kinetics, and radiation sensitivities of spermatogenic cells in the mouse. The LD₅₀ is the radiation dose necessary to kill 50% of the cells. Spermatids with sonication-resistant nuclei in the shape of sperm heads are indicated by the stippled bar.
which is the increase in numbers of stem cells, and 2) repopulation, which is the differentiation of the stem cells and the refilling of the tubules by their progeny.

In man, the process of spermatogenesis is almost identical to that in rodents; the type B spermatogonia, all stages of primary spermatocytes, secondary spermatocytes, and round and elongating spermatids have features analogous to those cells in the rodents (Clermont, 1966, 1972). The kinetics of differentiation of these cells in humans can be calculated by multiplying the times between the analogous stages in the mouse by 1.9, which is the ratio of the respective times for the cycle of the seminiferous epithelium in man (16 days) and in the mouse (8.6 days) (Oakberg, 1956; Clermont, 1972). However, the sequence of differentiation of the A spermatogonia, the identification of the stem cells, and the kinetics of stem cell renewal and regeneration have not been determined in man (Clermont, 1966).

**Effects of Anticancer Agents on Differentiating Spermatogonic Cells**

The effects of various anticancer agents on the spermatogonic process have been examined (Meistrich et al., 1972; Lu and Meistrich, 1979; da Cunha et al., 1985), using the following approaches to determine the most sensitive targets. All drugs were injected intravenously or intraperitoneally, in order to obtain relatively uniform distribution of a moderate dose of drug throughout the testis. Intratesticular injection yields high local concentrations, and, although interesting results have been obtained, effects on less sensitive cell types are often detected (Parvinen and Parvinen, 1978; Russell et al., 1983). In addition, effects were analyzed at least several days after treatment in order to allow dividing cells to attempt several mitoses: irradiated cells and those treated with some antineoplastic agents usually progress through their first mitotic division but die in subsequent cycles (Jung, 1982).

Spermatogonic cell killing was analyzed either by histologic examination of the testis or by counting sperm heads. Testes were prepared for histologic analysis 11 days after treatment with a cytotoxic agent; tubules at all stages of the seminiferous epithelial cycle were scored for the presence, reduction in numbers, or absence of the specific cell types that are supposed to be present at that stage. In these studies the missing cells were, almost without exception, of only one type, the primary spermatocytes. Based on the kinetics of spermatogenesis, these results indicate that the rapidly dividing A₁ through B spermatogonia are the most sensitive to cytotoxic agents. In the alternative method, sonication-resistant sperm heads in the testes were counted at various times after exposure (Amann, 1970; Mian et al., 1977). Sperm head counts reached minima at about 4 wk after radiation (Mian et al., 1977), Adriamycin (Hacker-Klom et al., 1985), or chlorambucil treatment, corresponding to the time required for differentiating spermatogonia to become late spermatids. The agreement of the two assay methods again confirms that most reproductive toxins do not alter the kinetics of spermatogenesis. Furthermore, the minimum in fertility is observed at 5–6 wk after treatment with radiation or some of the chemotherapeutic drugs (Searle and Beechey, 1974; Ehling, 1977), again confirming by a functional assay that the differentiating spermatogonium is the most sensitive target to this class of reproductive toxins. There are some exceptions, however; for example, high doses of procarbazine can cause abnormalities in spermatid development (Russell et al., 1983) as well as infertility at times at which treated spermatocytes and spermatids would have produced sperm (Lee and Dixon, 1972).

The target in the reproductive system that is most sensitive to the toxic effects of radiation and most cancer chemotherapeutic drugs seems to be the rapidly dividing differentiating spermatogonia. It is logical that these cells should be the major targets for such agents, which have been selected for their ability to kill rapidly dividing (i.e., tumor) cells.

**Effects of Anticancer Agents on Spermatogonial Stem Cells**

The stem cells are most important for the continued maintenance of the spermatogenic process; their killing results in long-term and possibly permanent sterility. Survival curves for stem cells following radiation have been obtained by direct counts of these cells in seminiferous tubule sections or whole mounts (Erickson, 1976; Oakberg, 1978). However, stem cell identification is difficult and time consuming, and the presence of a stem cell does not guarantee its subsequent viability and function. Alternatively, two functional assays have been developed to measure stem cell survival. The first involves counts of repopulating tubule cross-sections, usually at 5 wk after treatment (Withers et al., 1974). Each seminiferous tubule cross-section in a histologic preparation is scored for the presence or absence of spermatogonic cells; cross-sections showing repopulation with spermatogonic cells indicate the
presence of at least one surviving stem in that region. The second assay involves counts of sperm heads in the testis at 56 days after treatment; these sperm heads must have arisen from cells that were stem spermatogonia at the time of treatment (Lu et al., 1980).

The stem cells are more resistant to killing by antineoplastic agents than are the differentiating spermatogonia (Meistrich, 1984b), probably as a result of their low proliferative fraction. Several chemotherapeutic drugs are specific for killing S-phase cells, and therefore affect only very few of these stem spermatogonia. Radiation and other drugs damage DNA; however, there is sufficient time for DNA repair before the stem cells enter the critical phases of their cycle, at which time this damage is fixed or expressed.

Regeneration of the stem cells can be measured by giving two doses of the toxic agent and measuring the increase in numbers of surviving stem cells when the interval between doses is increased. In contrast to other renewing normal tissues (skin, intestinal epithelium, bone marrow) (Withers et al., 1974), there was no regeneration of the typically radiation-resistant mouse testicular stem cells during the first month following irradiation (Meistrich et al., 1978). The consequence of this observation that stem cell recovery is so slow is that damage from fractionated exposure to agents cytotoxic to stem cells will probably show some accumulation with multiple exposures.

In the mouse, following exposure to radiation or alkylating agents, surviving stem cells divide and begin repopulating the seminiferous epithelium with differentiating spermatogenic cells quite quickly (van Keulen and de Rooij, 1975; Huckins and Oakberg, 1978b), producing an appreciable number of sperm within 8 wk after exposure (Lu et al., 1980). Subsequent regeneration of stem cells results in an increase in the sperm production rate with time; however, after high doses it reaches a plateau value below that of controls. This plateau level of recovery of sperm production was compared to the number of surviving stem cells after a variety of radiation treatments, and a regression line was derived (Fig. 2a). The plateau level of recovery of sperm production after chemotherapy bears the same relationship to the initial stem cell survival as was the case with irradiation (Fig. 2b). The reasons for the incomplete repopulation of the seminiferous epithelium are not yet clear. It could be a result of homeostatic mechanisms limiting the regeneration of stem cells, induced damage in stem cells, or damage to the nongerminall cells of the testis, rendering them unable to support the same number of stem cells or rate of sperm production as before the toxic insult. Since the plateau level of return of sperm production is directly related to survival of stem cells for a variety of different agents, it is most likely that the cause of incomplete recovery would be a factor that limits the regeneration of stem cells; it is less probable that dam-

![FIG. 2. Relationship between recovery level of testicular sperm production and testicular cell survival in mice. The relative number of surviving stem cells (SSI) (abscissa) was determined from counts of repopulating tubules. Because of the correction for Poisson statistics, some values for the number of colonies per tubular cross-section can be greater than 1, although less than 100% of the tubules show repopulation. The plateau level of sperm head counts (SHC) (ordinate) was based on either the asymptotic value when the entire time course was determined or the counts at 44 wk after treatment were used. Each point represents one dose level of radiation or drug. (a) Points obtained by various radiation treatments: (●) single dose of gamma radiation; (●) 2 fractions of gamma radiation 24 h apart; (●) single dose of gamma radiation after giving the radioprotective drug WR-2721 (Meistrich et al., 1984); (●) single dose of irradiation with neutrons. The equation of the linear regression line is: ln (SHC) = 0.75 ln (SSI) −0.48. (b) Points obtained with various drug treatments given as single injections: (●) ADR, Adriamycin; (●) THIO, thiotepa; (●) BUS, busulfan. The regression line obtained for radiation is plotted again here for comparison with these points.](https://academic.oup.com/biolreprod/article-abstract/34/1/17/2764138)
age to some other target would be exactly proportion-
al to stem cell killing and hence result in such a good correlation for the various agents.

It is important to relate these effects to the ultimate end point for assessing the function of the reproductive system—fertility. After treatment with most antineoplastic agents, mice become infertile and experience a long sterile period before regaining their fertility. The time at which fertility returns is dose dependent and corresponds with the time sperm counts return to about 10% of control levels (Meistrich, 1982). The reciprocals of the times at which fertility returns are plotted as a function of stem cell survival in Fig. 3. The radiation data are used to calculate a regression line relating the duration of sterility to stem cell survival (Fig. 3a); the results obtained with chemotherapy fall very close to this regression line (Fig. 3b). Thus, the duration of sterility can be predicted solely from measurement of stem cell survival. Because the same relationship holds for such a variety of agents, stem cell killing and the resulting reduction of sperm production must be the primary causes of the long sterile period; it is unlikely that damage to any other target contributes significantly to the infertility observed after treatment with these agents.

In addition to a reduction in sperm production, anticancer agents can induce persistent alterations in the quality of sperm produced. Sperm head abnormalities and detachment of the sperm heads from the midpiece and tail have been observed in epididymal sperm samples taken from mice at long times after Adriamycin treatment (Meistrich et al., 1985). The target for this damage is unknown. It may be genetic damage fixed in the stem cells that gives rise to abnormal sperm (Wyrobek and Bruce, 1973) or damage to the Sertoli cells or other stromal elements of the testis. The poor quality of the sperm undoubtedly lowers the fertility potential of the animal; however, this effect is not significant enough to cause a deviation from the relationship between stem cell survival and duration of sterility (Fig. 3).

**Effects of Anticancer Agents on Spermatogenesis in Man**

The kinetics of response of the human germinal epithelium to anticancer agents is analyzed according to the principles developed from studies on rodents. As in mice, sperm count is maintained for several weeks following radiation with doses up to 300 rad (Heller et al., 1965). The counts reach very low levels at 8.5 wk, which is the time necessary in man for the differentiating spermatogonia to become spermatozoa. These results and histologic studies demonstrate that later spermatocytes and spermatids are more resistant to killing by irradiation than are type B spermatogonia (Rowley et al., 1974). A similar lag period before the decline of spermatogenesis begins appears to be

![Graph](https://academic.oup.com/biolreprod/article-abstract/34/1/17/2764138/figs/fig3.png)

**FIG. 3.** Relationship between time to recovery of fertility of male mice and testicular stem cell survival. The relative number of surviving stem cells (SSI) (abscissa) was determined from counts of repopulating tubules. The reciprocal of the median time of return to fertility is plotted on a linear scale (left ordinate); the corresponding actual numbers of days are also indicated (right ordinate). Each point represents one dose level of radiation or drug. (a) Points obtained with various radiation treatments. Symbols are as in Fig. 2a with the addition of (b) 16 fractions of gamma radiation given at 4-h intervals. The equation of the linear regression line is \((time)^{-1} = 0.00262 \ln (SSI) + 0.0102\). (b) Points obtained with various drug treatments and, for comparison, the regression line obtained with radiation.
the case with chemotherapeutic drugs (Richter et al., 1970). During continuous exposure or treatment with multiple courses of chemotherapy, azoospermia invariably occurs and is maintained. With such treatments, the overall sensitivity of spermatogenesis will be determined by that of the most sensitive stage, which for most anticancer agents is the differentiating spermatogonia.

Following termination of exposure to the toxic agent, spermatogenesis often recovers, but several different patterns of recovery may be observed (Fig. 4). In some cases, such as treatment with AMSA (4'-[(9-acridinylamino) methanesulfon-η-anisidide]), spermatogenesis recovers rapidly. Azoospermia occurred in a patient during chemotherapy with AMSA and continued for 3 wk after treatment; however, at 12 wk after treatment, which is sufficient time for stem cells to become sperm, the sperm count returned to a level not significantly different from pretreatment counts (da Cunha et al., 1982). The kinetics of decline and recovery of spermatogenesis following AMSA treatment is consistent with killing the differentiated but not the stem spermatogonia in man, as was observed to be the case in mice treated with AMSA (da Cunha et al., 1985).

Other agents induce permanent sterility, as documented by long-term follow-up of patients treated with six or more cycles of combination chemotherapy with the MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) regimen (Sherins and DeVita, 1973; da Cunha et al., 1984). At least 85% of the patients treated with this combination remain azoospermic. Histologic examination of testes of these azoospermic individuals in most cases reveals a complete absence of spermatogenic cells (van Thiel et al., 1972). In view of the ability of some of these agents (particular procarbazine) to kill testicular stem cells (Meistrich et al., 1972; Gould et al., 1983), we attribute the permanent azoospermia to the fact that the six cycles of MOPP chemotherapy is sufficient to kill all the testicular stem cells in man. It is not clear at what time after treatment the remaining 10–15% of the patients do recover spermatogenesis; stratification of the data by time after treatment does not indicate

**FIG. 4.** Models to explain the observed kinetics of decline and recovery of sperm production in humans treated for cancer with cytotoxic therapies.

<table>
<thead>
<tr>
<th>Observed Kinetics of Sperm Count Changes</th>
<th>Presumed Degree of Stem Cell Killing</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NONE</td>
<td>Surviving stem cells immediately repopulate seminiferous epithelium. Sperm production returns to normal.</td>
</tr>
<tr>
<td></td>
<td>SOME</td>
<td>Surviving stem cells do not begin repopulation until after a long lag period, which may correspond to the time required for stem cells to first regenerate their numbers. Recovery of sperm production may or may not be complete.</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>No stem cells remaining to repopulate seminiferous epithelium Permanent azoospermia.</td>
</tr>
</tbody>
</table>
any significant progressive recovery between 1 and 4 yr after treatment. We have observed some recovery of spermatogenesis at longer times (9yr) after treatment in only one patient (da Cunha et al., 1984).

A different pattern is observed following treatment with intermediate dose levels of radiation or chemotherapy. Azoospermia lasting for several years may be followed by recovery to pretreatment levels or possibly to a plateau level below that observed prior to treatment in individuals receiving testicular irradiation (Rowley et al., 1974; Clifton and Bremner, 1983; Berthelsen, 1984), three or fewer cycles of MOPP chemotherapy (da Cunha et al., 1984), or chlorambucil (Cheviakoff et al., 1973). Such extended periods of azoospermia preceding recovery are not observed in rodents because surviving stem cells immediately begin production of differentiating cells, which repopulate the seminiferous epithelium simultaneously with regeneration of stem cell numbers.

> Although it has not been possible to study directly the kinetics of stem cell regeneration and spermatogonial repopulation after cytotoxic insults to the human testis, the existing data on sperm counts can be used to develop the following model (Fig. 4). After the end of exposure to agents that are not cytotoxic to stem cells, regeneration of stem cells is not necessary and they can begin producing differentiated cells immediately. This results in the immediate resumption of sperm production as is seen after AMSA treatment. On the other hand, following exposure to agents that do kill an appreciable number of stem cells, a period of time is required for stem cell regeneration to occur. We suggest that in man some regulatory mechanism operates during this time to suppress the differentiation of the stem cells. If stem cell regeneration is quite slow, it might even take years before the differentiation process resumes. Histologic confirmation of this model is required, as is isolation of regulatory factors, which might be growth factors, differentiation factors, or chalones, that stimulate or inhibit the proliferation and differentiation of spermatogonial stem cells. There are reports demonstrating chalone activity in testicular extracts (Irons and Clermont, 1979; de Rooij, 1980); however, another laboratory failed to find such activity (Cunningham and Huckins, 1979). Further research is very important to obtain a better understanding of human spermatogenesis and to explain the observed toxicologic effects. Knowledge gained could be utilized to either repress stem cell proliferation during the time when exposure to agents that specifically act on proliferating cells would be expected, or perhaps to enhance proliferation after the end of the exposure period in order to speed up the recovery process.

Repression of stem cell proliferation to protect against chemotherapy-induced damage has been attempted both experimentally (Glode et al., 1981) and clinically (Johnson et al., 1985) by altering gonadotropin levels and action. However, the assumptions on which this approach is based do not appear to be valid for the following reasons: 1) the kinetics of spermatogenesis is not changed by altering hormonal levels, but rather sperm production may be reduced by degeneration of differentiating cells; 2) there is no evidence that spermatogonial proliferation is dependent upon gonadotropins or testosterone (Clermont and Harvey, 1965), but rather endocrine alterations produce effects at post spermatogonial stages; and 3) loss of differentiating spermatogenic cells might actually stimulate the undifferentiated spermatogonia into greater proliferative activity (de Rooij et al., 1985). A proper application of knowledge of the male reproductive system will be required for developing a method of preventing the toxic effects of agents on spermatogenesis.

Quantitative Effects of Reproductive Toxins

Until now, we have only discussed which cell types are the targets for reproductive toxicity; the next step involves determination of doses at which the toxicity and infertility actually occur. At high doses many agents may be reproductive toxins, but the ones of primary concern are those that would produce infertility at doses to which human populations might be exposed. The ability to predict toxic doses prior to the actual exposure of a human population, based only on dose-effect relationships observed in experimental animals, is of major importance. Anticancer agents constitute one of the few classes of agents for which some data are already available.

To test methods for interspecies extrapolation of the effects of toxin agents, dose-effect relationships must first be obtained in both experimental animals and man for some model toxins. These relationships will be compared by the use of an extrapolation factor (EF); the EF is defined as the dose necessary to produce a given toxic effect in the experimental animal divided by the dose to produce the same effect in man. If we could accurately measure the dose to the target organ or cell type, the EF would indicate the relative sensitivity of human and animal spermatogenesis to a given dose of reproductive toxin. How-
ever, because of differences in metabolic rates between small laboratory animals and the average 70-kg human, the determination of the equivalent target organ dose may be complex and may require more complete pharmacokinetic information. If only the administered dose is specified, any differences in how this dose is distributed, metabolized, and excreted in the different species will appear as a component of the EF.

In the quantitative examples presented here, I use as end points a given reduction in sperm count or permanent azoospermia. Although sperm count is not an absolute measure of fertility (except for azoospermia), it reflects the fertility of the treated mice; in man, it is currently the only measure that can be quantitatively related to the probability of being fertile (Meistrich and Brown, 1983). If other end points were available and related to fertility potential, they could also be used; their development is clearly a challenge to reproductive biologists.

Radiation is an excellent model agent for interspecies comparisons because radiation is delivered directly to target cells. Using as an end point the dose of X- or gamma radiation required to reduce sperm count by a factor of 2, we have calculated EFs from mouse to man (Meistrich and Samuels, 1985). It was striking that the EFs were very dependent upon the time chosen for assay. For example, at a time when spermatozoa should be derived from stem cells surviving irradiation (56 days in the mouse and an equivalent time of 115 days in man), the EF is 21, which is very high. In contrast, if sufficient time is allowed for maximal recovery of sperm production in both species, the EF is calculated to be near unity as follows. Up to 400 rad given as a single dose does not produce any permanent reduction in sperm production in man, with insufficient data being available at higher doses (Rowley et al., 1974; Clifton and Bremner, 1983). In the mouse, 700 rad is required to reduce sperm counts permanently to 50% of control levels, resulting in an EF of less than 1.7. These EFs, summarized in Table 2, indicate the importance of defining the time at which two species are compared. As indicated in Fig. 4, human spermatogenesis is probably more sensitive to radiation when analyzed at short times, because in man there appears to be an extended delay before the surviving stem cells initiate their differentiation, whereas in the mouse differentiation begins immediately. Thus, for other agents that also kill stem cells, man should display a much greater sensitivity than test animals at short times after exposure; however, if sufficient time is allowed for recovery, sensitivities should be similar.

To apply these concepts to chemical agents, it is important to consider the dose units used as the basis of the interspecies extrapolation. Although administered dose has traditionally been expressed on the basis of body weight, it has been found that interspecies extrapolation of the toxic effects of anticancer agents is more accurate on the basis of body surface area, or weight$^{2/3}$ (Freireich et al., 1966). Both methods of expression of dose will be used for calculation of the EFs and compared (Table 2).

Preliminary measurements of the doses of the alkylating agent chlorambucil and procarbazine (whose reactions with macromolecules is of a similar type to the alkylating agents) necessary to produce permanent azoospermia in the mouse have been obtained (Meistrich et al., unpublished observations). Extrapolation of these doses to man on the basis of body surface area better predicts the doses that produce permanent azoospermia in man (Cheviakov et al., 1973; Guesry et al., 1978; Marina and Barcelo, 1979; da Cunha et al., 1984) than does extrapolation based on weight (Table 2). In contrast, neither method

<table>
<thead>
<tr>
<th>Agent</th>
<th>End point</th>
<th>Extrapolation factor</th>
<th>Units of dose</th>
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<tbody>
<tr>
<td>X-rays</td>
<td>Reduction in sperm count by 50% at</td>
<td>21</td>
<td>rad</td>
</tr>
<tr>
<td></td>
<td>time stem cells become sperm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-rays</td>
<td>Permanent reduction of sperm</td>
<td>&lt; 1.7</td>
<td>rad</td>
</tr>
<tr>
<td></td>
<td>count to 50% of controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Permanent azoospermia</td>
<td>0.4</td>
<td>mg/m^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>Permanent azoospermia</td>
<td>&lt; 2</td>
<td>mg/m^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 23</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>Permanent azoospermia</td>
<td>&lt; 0.05</td>
<td>mg/m^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 0.6</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>
very accurately predicts the doses of Adriamycin that man can tolerate without incurring permanent azoospermia (Lu and Meistrich, 1979; Shamberger et al., 1981; da Cunha et al., 1983), but there appears to be less of a discrepancy when the doses are expressed on the basis of body weight. This may result from a different distribution of Adriamycin because of its higher lipophilicity. However, based on these examples, it is clear that further study of the intratesticular pharmacokinetics and cellular damage produced by these agents is necessary to determine the proper method for expressing the dose to the target tissue and cell and whether interspecies differences in the metabolism or response to certain agents exist.

Infertility Resulting from Reproductive Toxins

Although measurement of fertility of experimental animals is commonly used for testing potential reproductive toxins, it is not a very sensitive end point and cannot be used for quantitative determination of doses at which humans would be at risk for reproductive toxicity (Galbraith et al., 1983). One reason for this is that most experimental animals produce spermatozoa in vast excess of the number required for fertility. Doses of toxins that would produce equivalent declines in sperm counts in experimental animals and man would most likely have very different effects on fertility.

We have developed an approach for estimating the increase in infertility in the human population resulting from exposure to reproductive toxins based on reduction in sperm count (Meistrich and Brown, 1983). Either sperm counts measured in human populations exposed to reproductive toxins can be used or, if exposure has not occurred, the quantitative methods presented above could be used to extrapolate experimental animal data to predict the reduction in sperm count in human populations from given doses. A method referred to as the "two-distribution model" has been developed to calculate the decrease in human fertility resulting from a given reduction in sperm counts (Meistrich and Brown, 1983). The model is based on observations that distributions of sperm counts from men in infertile marriages are skewed toward lower counts than those from men considered to be fertile. Thus, although an individual cannot be defined as being fertile or infertile based on a sperm count, it is possible to use the ratio of the two distributions to determine the probability that an individual with a given sperm count will fall into the infertile category. Exposure to a toxic agent will shift the distribution of sperm counts in the population to lower values, and thus the risk of infertility for each individual in the population will be increased. Although only the probability of infertility can be determined for individuals, the incidence of infertility in the population can be calculated by averaging these probabilities. The results indicate that an additional 4% of couples will become infertile as a result of a twofold reduction of sperm count in the men (Meistrich and Brown, 1983; Meistrich, 1984a).

The assumptions and limitations of the two-distribution model have been presented (Meistrich and Brown, 1983). One assumption was that the same function that relates sperm count to infertility risk in a supposedly unexposed population also will apply to the population exposed to a toxic agent. This generalization will be true if infertility risk is primarily related to sperm count and not to other factors that may differ in the population before and after exposure to the toxic agent. The data presented above indicate that this hypothesis may be valid in the case of mice treated with anticancer agents. It will be the task of reproductive biologists to determine for which toxic agents this model would apply to other experimental animals and to man. If sperm count in exposed populations is not predictive of the risk of infertility, other characteristics of the semen or reproductive system should be applied to improve the prediction of fertility.

Concluding Remarks

Knowledge of reproductive biology can effectively be applied to understand the important toxicologic problems that mankind is facing in his rapidly changing environment. Anticancer agents are only one of the multitude of reproductive toxins to which human males are exposed. Knowledge of normal spermatogenesis, particularly the kinetics of the process, is necessary in order to understand the action of anticancer agents and related environmental toxins. Further study of factors regulating the kinetics of stem cells is important for understanding the action of these agents on spermatogenesis.

However, there are many other agents that act on different targets to inhibit spermatogenesis and male fertility potential, and additional techniques must be applied to understand mechanisms toxicity. In addition to those that kill the spermatogonia, there are toxins that kill or alter the development of later stage
spermatocytes or spermatids. Biochemical study of separated cell populations (Meistrich, 1977) appears to be a useful approach to understanding the mechanism of action of reproductive toxins that act on these cell stages. Substances that perturb the endocrine system are also particularly important because they will affect spermatogenesis as well as other aspects of the reproductive process. Histologic techniques, employing good preparations for light or electron microscopic analysis, can be valuable for identifying toxins that act via the endocrine system; a consistent pattern of degeneration of cells at Stage VII of the cycle of the seminiferous epithelium is observed in rats treated with substances that are hormonally active or antagonistic (Russell et al., 1981). Another sensitive target whose alteration affects fertility potential is the motility of the spermatids. Methods for objectively measuring sperm motility (Katz and Overstreet, 1979) are essential for studies of toxic effects of environmental agents on this aspect of the reproductive process.

Finally, emphasis must also be placed on the quantitative methods for determining the reproductive toxicity to man resulting from exposure to a given dose, based on experimental animal data and other available information. This information is essential for assessing risk, determining risk/benefit ratios, and making regulatory decisions. To obtain such information, reproductive biologists must be aware not only of the basic mechanisms involved in the reproductive system, but also of problems of reproductive toxicology, and must focus their basic research to provide answers to problems that are occurring in actual human populations.

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