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Mechanism of bladder damage and repair after treatment with radiation and cytostatic drugs

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In this chapter the structure and tissue organization of the mammalian bladder is examined in relation to its major functions; i.e. expansion to accommodate large volumes of urine, micturition and maintenance of an impermeable barrier to water and small ions.

The time of expression of functional damage after surgical or chemical injury, or irradiation, is correlated with the time of onset of histological damage and with increases in the rate of epithelial cell proliferation. The extent to which normal function is regained after different damaging agents is compared.

Structure and function of the bladder

The mammalian bladder consists of a mucosa with 3 to 5 cell layers of transitional epithelium, fibrous connective tissue containing the blood vessels and nerve fibres, and three smooth muscle layers. The epithelium is arranged in a pattern of increasing differentiation from small, diploid, basal cells to the large, specialized, surface cells which are always polyploid and sometimes binucleate.

Associated with the muscle layers are three sphincters which are responsible for the maintenance of continence and which allow accumulation of urine beyond the point where the bladder would reflexly void. The process of micturition is initiated through sensory impulses in the bladder wall which are conducted via afferent tracks of the parasympathetic nerves S_2, S_3 and S_4. This causes efferent responses, through the same nerve tracks, and results in muscular contraction which alters the shape of the bladder base and allows the urethra to open (Hutch, 1972).

The function of the mammalian bladder is to collect and store urine (without change in its chemical composition and without dilution) until it can be conveniently voided. In man a normal bladder is usually allowed to fill to approximately 300 ml although much larger volumes can be accommodated without damage. One major requirement of the bladder is therefore the ability to expand to hold increasing volumes of urine. Another important feature of the bladder mucosa is its relative impermeability to both water and small ions (Hicks, 1966; Hicks, 1969; Hicks *et al.*, 1974). The ionic composition and tonicity of urine are very different from that of the blood, which lies only a few microns below the luminal surface and yet urine remains in the bladder for many hours without dilution or change in its chemical composition (Johnson *et al.*, 1951; McIntyre & Williams, 1969). No other epithelium is capable of acting as such an efficient barrier to the passage of water and in cases where artificial bladders have been constructed (from ileal or rectal epithelium) reabsorption of nitrogenous wastes and loss of body water occurs (Marucci *et al.*, 1954; Blandy, 1964).

It is the highly specialized nature of the superficial cells lining the bladder lumen which confers both the ability to expand in volume and to restrict free passage of water and small ions between blood and urine (Hicks, 1966; Koss, 1969). The surface cells are extremely large and cover up to 20 underlying, intermediate cells when the bladder is distended (Walker, 1958; Levi *et al.*, 1969*b*; Cooper, 1972). The plasma membrane of these cells is asymmetric with the luminal surface being composed of thick hexagonal plaques (100–120 Å), separated by thinner, 'hinge' areas (Hicks, 1965; Koss, 1969; Porter *et al.*, 1967). When the bladder contracts the membrane folds at the hinge areas causing an invagination of the luminal membrane to form fusiform vesicles (Hicks, 1966; Hicks, 1975; Porter *et al.*, 1967). This stored membrane is reinserted into the luminal surface as the bladder expands and the surface cells stretch. Membrane invagination is thought to be the result of physical contraction of the bladder rather than an autonomous, energy consuming process, since there are relatively few mitochondria associated with the membrane (Hicks, 1965; Hicks, 1966; Hicks *et al.*, 1974).

In addition to its unique morphology, the luminal membrane of the superficial bladder cells has an unusual chemical composition. The lipid component has a high proportion of cerebroside which may have an important function in maintaining impermeability (Ketterer *et al.*, 1973; Hicks *et al.*, 1974). Hicks (1965) has also suggested that keratin is incorporated into the thickened regions of the membrane, thus further decreasing

permeability. The presence of tight junctions between adjacent surface cells restricts the passage of small ions which generally travel via an intercellular route rather than transcellularly (Fellows & Marshall, 1972; Koss, 1969; Hicks, 1966).

Cell populations and kinetics of the bladder

The bladder mucosa is a polyploid tissue in which polyploidy is established early in embryonic life and does not increase significantly with ageing of the animal (Walker, 1958; Levi *et al.*, 1969*a*, *b*; Farsund, 1975). The DNA content of the epithelial cells increases from basal cells (2n) to surface cells. Surface cells in rodent bladders are frequently octoploid (8n) and sometimes even higher ploidy levels occur (Levi *et al.*, 1969*b*; Farsund, 1976), although in man octoploid cells are less common (Levi *et al.*, 1969*a*). It has been argued that polyploidy is established by upward migration and fusion of the diploid cells (Martin, 1972; Cooper *et al.*, 1972), but more recent evidence suggests that these cells are formed by repeated DNA synthesis without nuclear division (Farsund & Dahl, 1978; Locher & Cooper, 1970). Cells of all ploidy levels retain the ability to divide and mitotic figures have been observed even in octoploid surface cells (Levi *et al.*, 1969*b*; Cooper *et al.*, 1972), although cell division usually occurs in the diploid basal cells (Locher & Cooper, 1970; Martin, 1972; Farsund, 1976).

Under normal, unstimulated conditions the bladder epithelium has an extremely slow cell turnover rate (Levi *et al.*, 1969*b*; Schreiber *et al.*, 1969; Martin, 1972; Farsund, 1975; Stewart *et al.*, 1980). Pulse labelling with a single injection of tritiated thymidine ([^{3}H]-TdR) gives values of $<1\%$ for the labelling index (LI) of rodent bladders (see Table I for a summary of published values). Mitotic figures are so rare as to make an estimate of the mitotic index (MI) unreliable.

Changes in cell populations and cell kinetics after injury

Despite its normally slow rate of prolieration the bladder epithelium is capable of very rapid division in response to injury. Surgical wounding, mechanical stress, chemical injury and irradiation have all been shown to markedly increase the proliferative activity in the bladder (Walker, 1959; Zhuravliev, 1963; Bonser & Clayson, 1964; Dahioev *et al.*, 1969; Levi *et al.*, 1969*b*; Schreiber *et al.*, 1969; Koss & Lavin, 1970; Locher & Cooper, 1970; Levi *et al.*, 1971; Farsund, 1976; Farsund, 1977; Stewart *et al.*, 1980), although the time at which increased proliferation occurs depends upon the type of injury.

Acute injury after surgical incision (Walker, 1959) induces an immediate wave of proliferation in cells adjacent to the wound within 24 h. Chronic mechanical trauma produced by implantation of a glass bead in the bladder (Locher & Cooper, 1970) has also been shown to induce rapid cell division.

Chemical trauma by alkylating agents such as cyclophosphamide causes an initial period of cell depletion which first affects the specialized, octoploid surface cells (Farsund, 1976), thus exposing the deeper, non specialized epithelium. This is followed by a wave of increased proliferative activity initiated in the diploid cells within 2 to 4

Table I Proliferation parameters for unstimulated rodent bladder epithelium

		Turnover time (days)	
Author	*LI(%)*	*from* $T=\lambda T_s/LI$	*from continuous labelling*[a]
Stewart *et al.*, 1980 (mouse)	0.14	206	314 ± 113
Levi *et al.*, 1969 (mouse)	1.0	42	—
Schreiber *et al.*, 1969 (rat)	0.12	240	—
Locher & Cooper, 1972 (rat)	<0.1	>350	—
Martin, 1972 (guinea pig)	1.3	35	—
Farsund, 1975 (mouse)	0.4	105	—

[a]Mean value (± 1 s.d.) obtained from linear regression analysis and extrapolation to 100% labelling. T = Cell turnover time. λ = Correction for non-linear age distribution of cells around the cell cycle; assumed 0.693. T_s = DNA synthesis time; assumed 10 h. (Modified from Stewart *et al.*, 1980).

days and later involving cells of all ploidy levels (Locher & Cooper, 1970; Farsund, 1976; Koss & Lavin, 1970; Stewart, 1985). One notable feature of the regenerating bladder epithelium after cyclophosphamide injury is the production of cells of extremely high ploidy, e.g. up to 64n (Locher & Cooper, 1970). Some authors report a complete recovery of the bladder epithelium (with re-establishment of the tetraploid and octoploid cell populations) within 2 weeks of a single dose of cyclophosphamide (Philips *et al.*, 1961; Koss & Lavin, 1970), but there are other reports of a much slower recovery, with epithelial abnormalities and increased proliferative activity still seen at 2–3 months (Locher & Cooper, 1970; Stewart, 1985).

Carcinogenic agents such as 4-ethylsulphonyl-naphthalene-1-sulphonamide (Dzhioev *et al.*, 1969; Levi *et al.*, 1969*b*; Cooper *et al.*, 1972; Levi *et al.*, 1971) and dibutylnitrosamine (DBN) (Farsund, 1977) have also been shown to induce epithelial cell proliferation in the bladder. Farsund (1977) reported that DBN causes damage primarily to diploid, basal cells and that DNA synthesis is blocked for a period of 6 h immediately after administering the drug. He suggested that this agent may permanently damage the mechanism for repeated DNA synthesis without nuclear division, thus preventing the formation of new polyploid cells once the DNA synthesis block is released. It seems, therefore, that whereas cyclophosphamide causes damage to the bladder by direct contact of a toxic metabolite in the urine with the superficial epithelial cells, DBN primarily affects the diploid basal cells (presumably via the blood stream) whilst they are in DNA synthesis (Farsund, 1977; Philips *et al.*, 1961).

The time at which stimulated proliferation occurs after radiation damage is very different from that occuring after chemical or surgical injury. After irradiation most cell types do not die until they reach mitosis and attempt to divide. If one assumes that the stimulus for compensatory proliferation in a tissue is cell death (Denekamp, 1975; Lamerton, 1966) then the onset of proliferation in a slow turnover tissue such as the bladder should be delayed. Figure 1 illustrates the results of a continuous labelling experiment in mouse bladder with [^{3}H]-TdR given at 1, 3, or 9 months after a single radiation dose of 25 Gy. There was no stimulated proliferation at 1 month and only a little at 3 months but a large increase in LI was observed at 9 months. Such a long delay before the onset of proliferation is in marked contrast with the situation which occurs after cyclophosphamide injection (see Figure 2), which induces some increased proliferation within a week and a maximum response within 1 month.

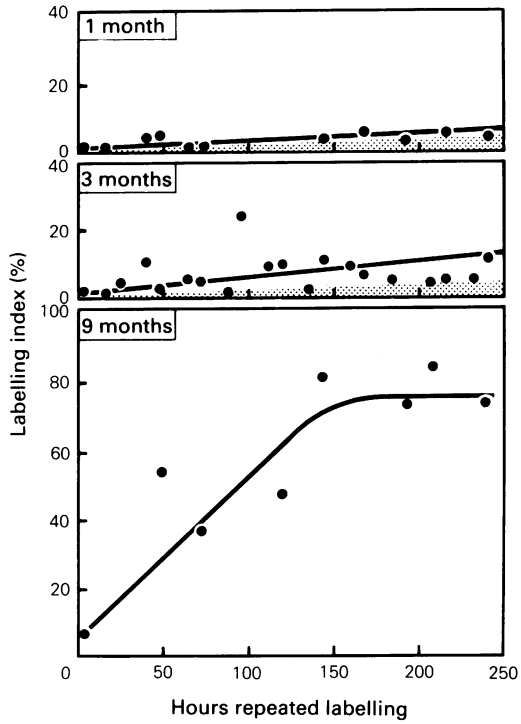

Figure 1 Continuous labelling data for mouse bladder epithelium at 1 to 9 months after irradiation with 25 Gy electrons. The LI is plotted as a function of time of exposure to [^{3}H]-TdR. Hatched areas indicate the very low rate of uptake of [^{3}H]-TdR in controls. (Data from Stewart *et al.*, 1980 and Stewart, 1985).

Zhuravliev (1963) found no evidence for stimulated proliferation in rat bladder within 2 months of irradiation (30 Gy), although the MI of control bladders was usually high (0.8%) in these experiments. This high rate of mitotic activity was probably due to the presence of a parasitic roundworm, Trichosomoides Crassicauda, which has been shown to stimulate proliferation in the bladder by inducing chronic irritation (Schreiber *et al.*, 1969). Results from the studies of Schreiber *et al.* (1969) on parasite free rats are, however, in conflict with those of Zhuravliev (1963) and Stewart & coworkers (Stewart *et al.*, 1980; Stewart, 1985) since they demonstrate an early increase in the LI (from 0.1 to 6%) at 1 week after irradiation (20 Gy), with a return to control values by 2 weeks. Neither Schreiber *et al.* (1969) nor Zhuravliev (1963) followed the proliferative pattern in the bladder at longer times (>3 months) after treatment and it is possible that much greater increases in proliferative activity would have been observed in their animals at periods of 6 months or more.

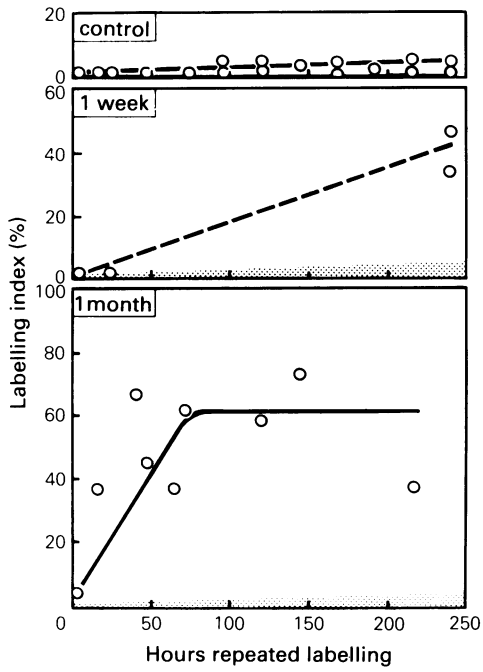

Figure 2 Continuous labelling data for normal mouse bladder epithelium (top panel), and 1 week or 1 month after a single dose of 75 mg kg^{-1} cyclophosphamide. (Data from Stewart, 1985)

A summary of some of the major findings relating to proliferation rates of the regenerating bladder epithelium after injury with cyclophosphamide or radiation is given in Table II.

Assays for measurement of function

Three functional assays have been used to quantify bladder damage: (a) permeability to water and small ions, (b) urination frequency, (c) bladder volume capacity under applied pressure.

(*a*) *The permeability* of dog, rat and rabbit bladders has been measured by the use of lithium or radiolabelled tracers (e.g. Johnson *et al.*, 1951; Hlad *et al.*, 1956; Turnbull, 1973; Hicks *et al.*, 1974). Permeability studies have also been carried out in patients with a history of either bladder cancer or urinary infection (Fellow & Marshall, 1972). In animal studies on bladder permeability the ureters are cut between ligatures and the bladder cannulated for instillation of the radionuclide. The rate of loss of isotope from the bladder can then be followed by external counting (Turnbull, 1973; Turnbull & Fellows, 1972). Alternatively, an i.v. injection of tritiated water (THO) is given and the accumulation of activity in the bladder monitored (Turnbull & Fellows, 1972). In

Table II Proliferation of mouse bladder epithelium after radiation or cyclophosphamide

Treatment	*Author*	*LI*(%)	*Turnover time (days)* from $T=\lambda T_s/LI$	*Turnover time (days)* from continuous labelling[a]
Radiation:				
25 Gy/1 mo	Stewart, 1985	0.9	32	239±182
25 Gy/2 mo	Stewart, 1985	0.4	72	123±59
25 Gy/3 mo	Stewart, 1985	1.1	26	65±45
25 Gy/6 mo	Stewart *et al.*, 1980	6.7	4	9±2
25 Gy/9 mo	Stewart *et al.*, 1980	6.2	5	9±3
25 Gy/12 mo	Stewart *et al.*, 1980	5.1	6	9±4
20 Gy/8 d	Schreiber *et al.*, 1969	6.4	5	—
8 Gy/3 d	Schreiber *et al.*, 1969	2.3	13	—
8 Gy/8 d	Schreiber *et al.*, 1969	0.4	72	—
Cyclophosphamide:				
75 mg kg^{-1}/7 d	Stewart, 1985	0.1	289	—
75 mg kg^{-1}/14 d	Stewart, 1985	0.2	144	—
75 mg kg^{-1}/1 mo	Stewart, 1985	5.1	6	8±2
75 mg kg^{-1}/2 mo	Stewart, 1985	2.1	14	10±2
75 mg kg^{-1}/3 mo	Stewart, 1985	5.4	5	11±7
200 mg kg^{-1}/2 d	Farsund, 1976	14.1	2	—
100 mg kg^{-1}/2 d	Locher & Cooper, 1970	5.9	5	—
100 mg kg^{-1}/4 d	Locher & Cooper, 1970	16.9	2	—
100 mg kg^{-1}/7 d	Locher & Cooper, 1970	3.1	9	—
100 mg kg^{-1}/42 d	Locher & Cooper, 1970	0.1	289	—
100 mg kg^{-1}/70 d	Locher & Cooper, 1970	<0.1	>289	—

[a]Mean value (±1 s.d.) obtained from linear regression analysis and extrapolation to 100% labelling. T=Cell turnover time. λ=Correction for non-linear age distribution of cells; assumed 0.693. T_s=DNA synthesis time; assumed 10 h.

patients, bladder permeability can be assessed from the amount of ^{24}Na retained in the body after the bladder has been emptied and rinsed (at 1 h after instillation of the isotope). Accumulation of tritiated water in bladders after i.v. injection has also been used to measure permeability to water in humans (Fellows & Marshall, 1972).

The rate of transfer of inorganic ions across the bladder is normally very low, i.e. $<0.5\%$ min^{-1}, (Hlad *et al.*, 1956; Levinsky & Berliner, 1959) and depends on the presence of intact tight junctions between adjacent superficial cells. The permeability to sodium and lithium increases by factors of up to 30 when these junctions are chemically broken (Turnbull, 1973; Hicks, 1966). Urinary infection or the presence of an undifferentiated bladder tumour also increases permeability to sodium in man (Fellows & Marshall, 1972). Hicks reports that the whole bladder acts as a passive barrier to the passage of water and that this barrier is dependent on an intact luminal membrane in the superficial cells (Hicks, 1966). However, in other experiments (Turnbull & Fellows, 1972) no association was demonstrated between an intact luminal membrane and permeability to water.

(b) *Urination frequency* has been used to measure bladder damage in mice after irradiation or cyclophosphamide (Stewart *et al.*, 1978; Stewart, 1985). Since there is a strong diurnal pattern of urination in mice (as in most other mammals) frequency measurements should be made over a 24 h test period. Measurements are made by housing mice in individual cages with wire bar floors beneath which absorbent paper is drawn at a speed of $\sim 15\,cm\,h^{-1}$ (Stewart *et al.*, 1978). Urination frequency is calculated from the number of discrete urine spots on the paper. An estimate of the urine volume produced per mouse can be made from the total *area* of urine spots if these are compared with a calibration curve constructed using known volumes of urine.

In normal, healthy mice both the 24 h volume and frequency of urination remains fairly constant (1–2 ml urine per 24 h, excreted as 8–12 separate urination events). After damage to the bladder by radiation (Stewart *et al.*, 1978; Stewart *et al.*, 1981) or cyclophosphamide (Stewart, 1985) the volume output per day is unchanged, except after very high doses where the general health of the animal is compromised, or where secondary renal failure occurs. The *frequency* of urination, however, increases in proportion to the amount of damage incurred. Urination frequency can either be expressed simply as the number of urination events per test period or as the number of events ml^{-1} of urine produced. This latter method is probably preferable since it takes account of any variations in 24 h urine volumes.

(c) *The capacity* of irradiated mouse bladders has been measured by inflating with air at pressures of 5 to 40 mmHg (Stewart *et al.*, 1981). For this technique the bladders are exposed (whilst *in situ*) immediately after killing the mice, a catheter inserted and the bladder size measured in three dimensions at increasing inflation pressures. In untreated mice the bladder volume increases from $\sim 100\,mm^3$ to $400\,mm^3$ as the applied pressure is increased from 5 to 35 mmHg (see Figure 3). Radiation induced fibrosis causes a decrease in the bladder volume for a given applied pressure. These effects begin at around 6 months but are not severe until 12 months.

Time of expression of damage

The time course for histological expression of damage after different chemical agents has been described briefly above. The *functional* response

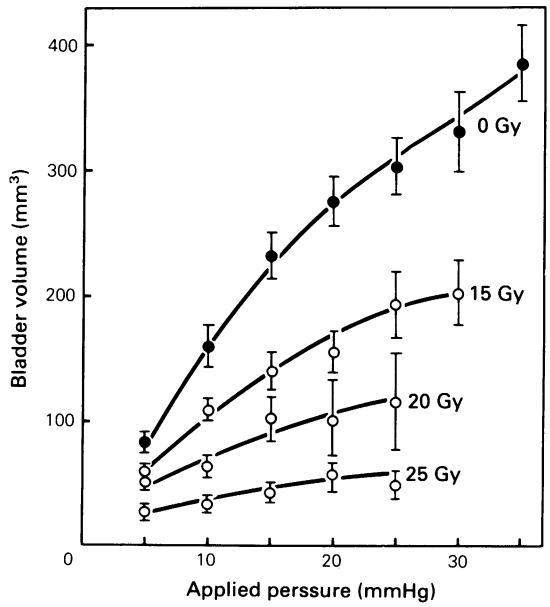

Figure 3 Mouse bladder volumes at increasing applied pressure. Measured at 15 months after treatment with 0 to 25 Gy electrons. (Data from Stewart *et al.*, 1981)

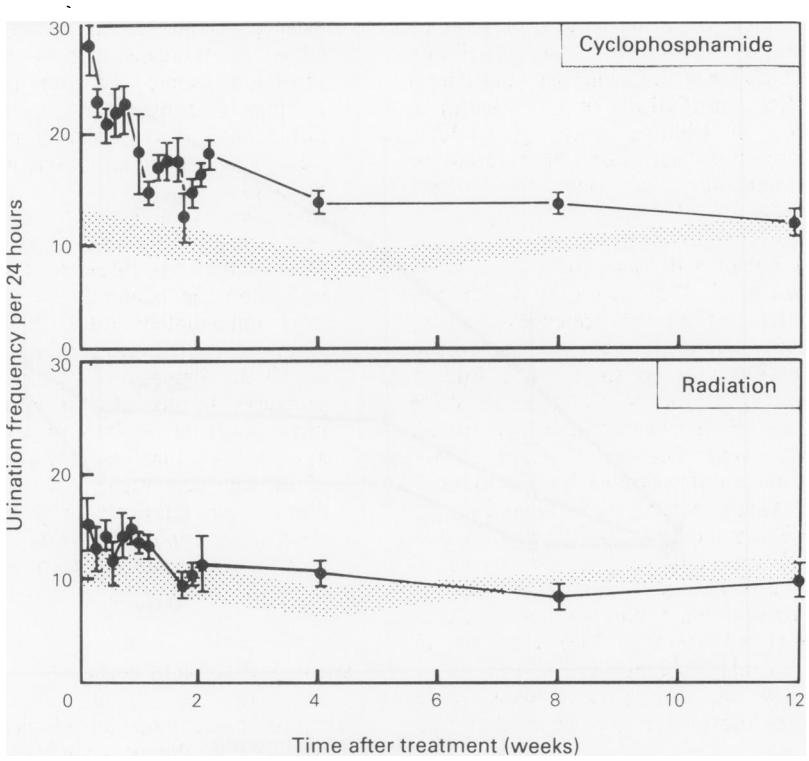

Figure 4 Mean urination frequency response for groups of mice (± 1 s.e.) during the first 12 weeks after treatment with $75\,mg\,kg^{-1}$ cyclophosphamide (top panel) or 32.5 Gy electron irradiation (bottom panel). The hatched area indicates response of controls during the same period. (Data from Stewart, 1985)

of mouse bladders to single doses of cyclophosphamide has also been measured using the urination frequency assay (Stewart, 1985). Within 1 day of a single dose of $75\,mg\,kg^{-1}$ cyclophosphamide the urination frequency increases by a factor of ~ 3 (see Figure 4). Urination frequency remains high for 8 weeks with a return to control levels after 3–5 months. The early time at which functional damage is seen agrees well with the rapid appearance of histological damage (epithelial denudation) after cyclophosphamide.

This is in marked contrast with the time of expression of damage after radiation, when there is no increase in urination frequency until at least 6 months (see Figures 4 & 5). From 6–9 months onwards there is a dose dependent increase in frequency with 30 or more urinations per day occuring in animals treated with the highest radiation doses. The time of onset of the functional damage again coincides with the appearance of epithelial disturbances such as the loss of surface cells and development of hyperplasia (see Figure 6). Until 6 months after irradiation the bladder epithelium in mice retains its basic three layered structure, with a well defined basal layer, an intermediate layer, and large superficial cells lining the bladder lumen. Some subcellular damage is, however, evident in the epithelial cells before 6 months (oedematous cytoplasm and large lysosomes), as well as mild changes in the blood vessels of the submucosa (Antonakopoulos *et al.*, 1984). By 9 months there is some epithelial hyperplasia with a loss of superficial cells and at 12 months severe hyperplasia which is usually focal and interspersed with areas of denudation. Gross disorganization and vacuolation also occur in the bladder at this time and no normal surface cells are present (see Figure 6).

These observations agree well with results of Antonakopoulos *et al.* (1984) in a study of the histology of irradiated rat bladders. Two other authors, however, (Hueper *et al.*, 1942; Zhuravliev, 1963) reported a much earlier development of severe changes in the epithelium, with desquamation and cystic hyperplasia occurring within a few months of a low radiation dose (3×4 Gy) in dog bladders (Hueper *et al.*, 1942), and degenerative changes seen within a few *days* of a

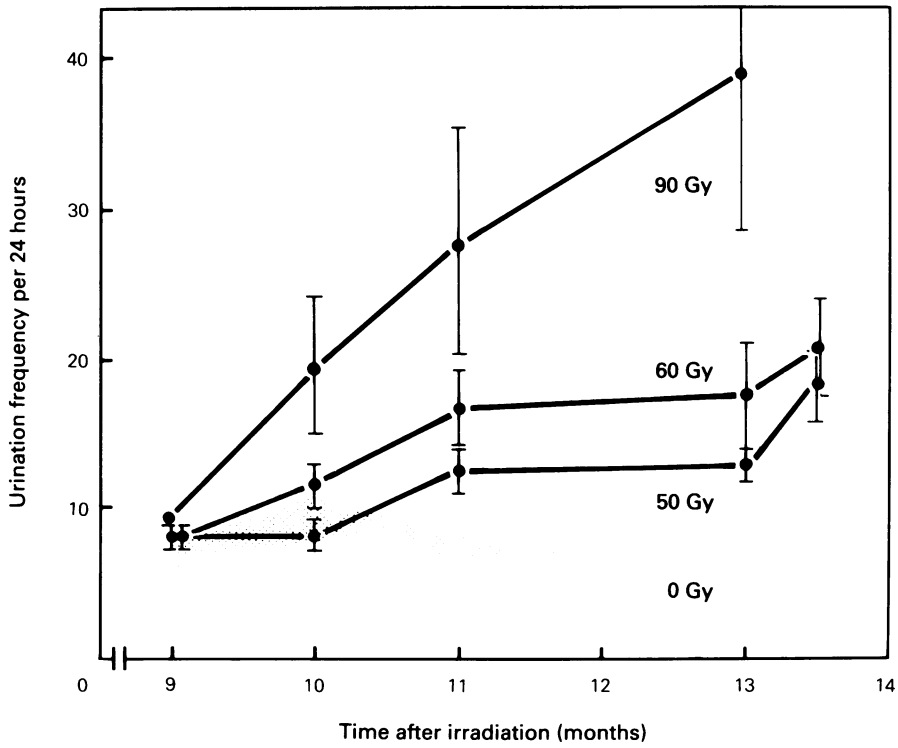

Figure 5 Mean frequency response for groups of mice tested from 9 to 14 months after irradiation with 20 fractions of electrons. The total irradiation doses are given beside each curve and the response of control animals is indicated by the hatched area. (Data from Stewart *et al.*, 1984)

single dose of 30 Gy in rats (Zhuravliev, 1963). The rat bladders were known to be infested with a parasitic roundworm which may have aggravated and precipitated the radiation response but no explanation can be found for the early appearance of damage in dog bladders. Such an early degenerative response of the epithelium is also sometimes observed in humans (e.g. Watson *et al.*, 1947).

In our own studies (Stewart *et al.*, 1980) and those of Antonakopoulos *et al.* (1984) normal surface cells were never re-established during an 18 month period after radiation doses of 25 Gy or more. This contrasts with the situation seen after damage with cyclophosphamide or mechanical wounding where surface cells are replaced at the time of the compensatory regenerative response (Walker, 1959; Koss, 1969; Farsund, 1976).

The onset and rate of development of radiation damage in the bladder is dose dependent, with more rapid expression of damage after the highest doses (Stewart *et al.*, 1978; Stewart *et al.*, 1984*a*; Figure 5). Functional radiation damage appears to be persistent, with little recovery even after 18 months. Whether the late functional impairment (increased urination frequency) is due to a failure of the regenerating epithelium to produce normal superficial cells, or whether it is due to developing fibrosis and a reduced bladder capacity, is unclear.

Radiation dose-response relationships

(a) *Repair in fractionated treatments.* Both urination frequency and reduced bladder capacity have been used to quantify the extent of damage in mouse bladders after treatment with fractionated irradiation (Stewart *et al.*, 1981; Stewart *et al.*, 1984*a*). Examples of dose effect curves after 1, 5, 10 or 20 doses of irradiation are shown in Figure 7. The amount of repair of sublethal injury which occurs between fractions can be estimated by comparing the *total* isoeffective doses for the different fractionation schedules. The bladder clearly has a large capacity for repair of sublethal radiation damage with a total dose of ~70 Gy in 20 fractions being equivalent to a single dose of 25 Gy. Repair in the bladder appears to be slightly more extensive

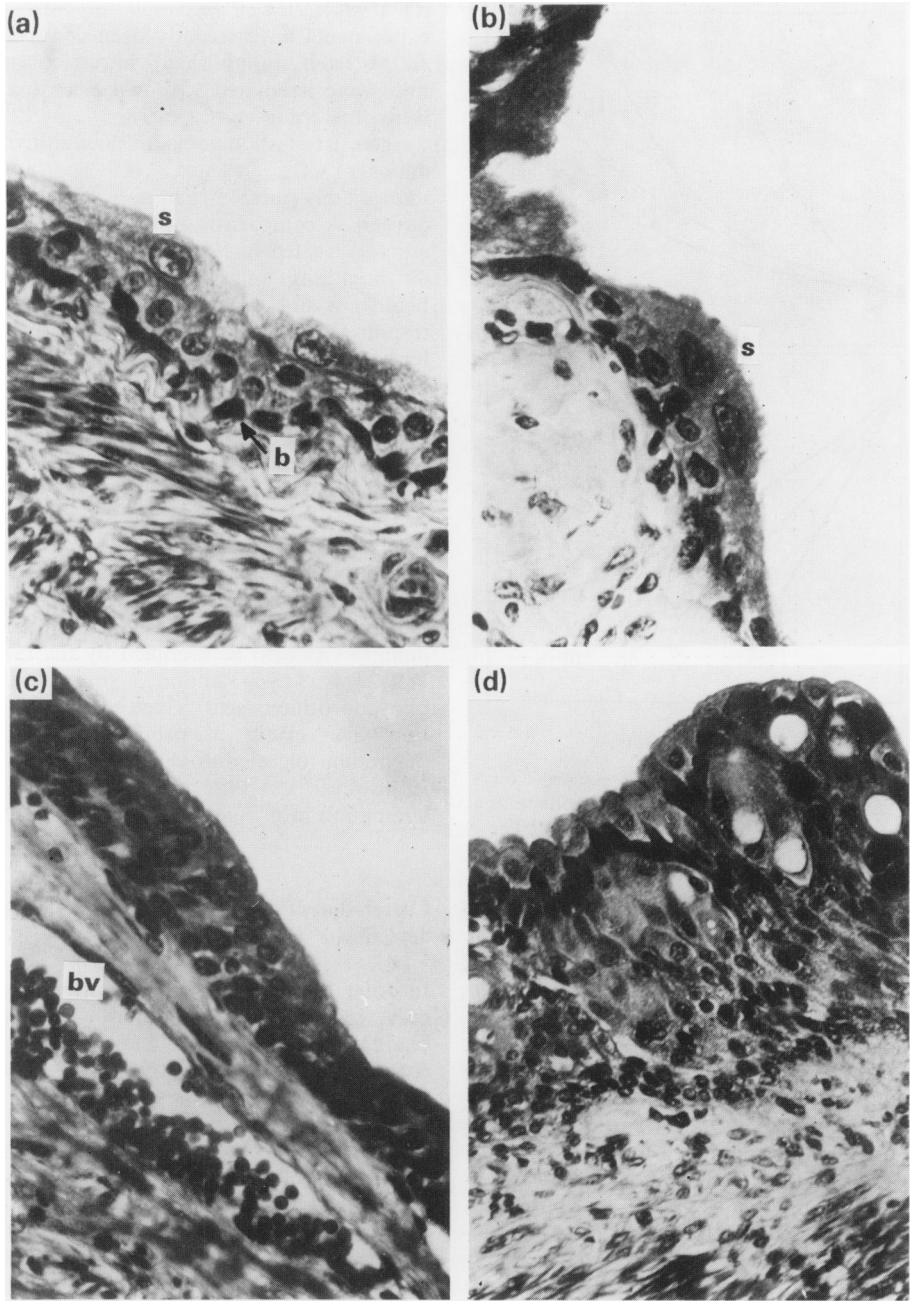

Figure 6 Epithelium and submucosa of mouse bladder:

a) Control. Note the well defined basal layer with darkly staining basophilic cells (b) and the presence of 2 large surface cells (s).

b) 3 months after 25 Gy. The epithelium is essentially intact. A normal, binucleate surface cell is clearly visible (s).

c) 9 months after 25 Gy. Mild hyperplasia in the epithelium with no normal surface cells. Enlarged blood vessels (bv) can be seen in the submucosa.

d) 12 months after 25 Gy. Marked hyperplasia with cellular vacuaolation. Normal surface cells are absent.

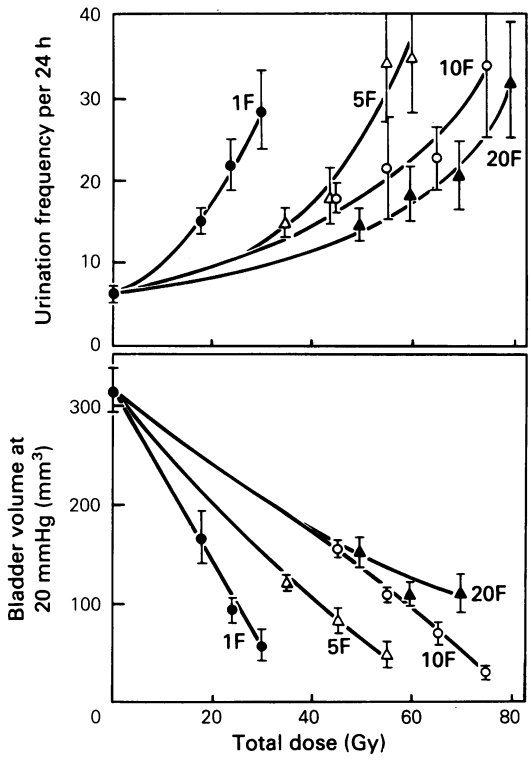

Figure 7 Radiation dose response curves for urination frequency (top panel) or bladder volume at an inflation pressure of 20 mmHg (bottom panel). The mean response for groups of mice tested at 11 to 14 months after 1 to 20 fractions of electrons is shown. (Data from Stewart *et al.*, 1984)

than in skin, although tissues such as lung and kidney have an even greater capacity for repair of sublethal damage after multiple, small radiation doses (Stewart *et al.*, 1981; Stewart *et al.*, 1984*b*; Travis *et al.*, 1983; Parkins *et al.*, 1985; Thames *et al.*, 1982).

(b) *Repopulation.* Since the bladder is a slow turnover tissue with a delayed response to irradiation (see above), little contribution to tissue recovery would be expected from repopulation during a protracted course of radiotherapy. Experiments in which 2 fractions of radiation were given in total treatment times of 1 day, or 1, 2 or 4 weeks, did not demonstrate any additional dose sparing for the longer time intervals (Stewart *et al.*, 1981). This is entirely consistent with data from continuous labelling studies (see above and Stewart *et al.*, 1980), which demonstrate a delayed onset of compensatory proliferation in the bladder until 6 months after irradiation.

(c) *Modification of radiation response.* A series of experiments have recently been completed (Stewart & Michael, unpublished) in which anaesthetized mice were irradiated with fast electrons whilst they were breathing nitrogen or 7%, 50%, or 95% oxygen. Irradiation took a maximum of 40 sec and animals were 'rescued' with an oxygen flush immediately after irradiation in nitrogen or 7% oxygen. A comparison of the urination frequency of animals treated in 50% oxygen with the response of air breathing animals (Figure 8) shows that the bladder is not normally fully oxygenated since the response can be increased by increasing the oxygen tension. Irradiation in 7% oxygen produced a slight decrease in sensitivity and in nitrogen the bladders were very radioresistant with an Oxygen Enhancement Ratio (OER) (obtained from a comparison of the 95% O_2, data not shown, or 50% O_2 and nitrogen dose effect curves) of 2.5 to 2.7.

The radiation sensitivity of the bladder can similarly be increased by irradiating in the presence of the radiosensitiser misonidazole (Figure 9), confirming that the target cells for radiation damage are not normally fully oxygenated. Bladder damage can also be modified by the radioprotector WR-2721 (S-2-3-amino propylamino ethyl-phosphorothioic acid) given 30 min before irradiation. The extent of protection afforded by the maximum tolerated dose of WR-2721 (400 mg kg^{-1}) is, however, less than the protection observed after irradiation in N_2 (compare Figures 8 & 9).

Correlation of radiation injury with target cell depletion

In order to establish which are the important target cells responsible for radiation damage in the bladder a comparison of the time of occurrence of pathological and histological changes with functional impairment must be made. Clinically, some degree of bladder irritation and pollakiuria (increased frequency) is often observed during radiotherapy treatment. This reaction is always transient, usually mild, and is probably the result of inflammation, either with or without the presence of bacterial infection (Watson *et al.*, 1947; Dean, 1927; Dean, 1933; Morrison & Deeley, 1965). Severe complications as a result of bladder irradiation do not occur until many months or years after treatment. These complications include frequency, dysuria, haematuria (sometimes with severe haemorrhage), contracted, fibrotic bladders (this condition is often difficult to distinguish from recurrent disease), and occasionally the development of fistulae (Dean, 1927; Everett & Baltimore, 1934; Gowing, 1960; Morrison & Deeley, 1965). The

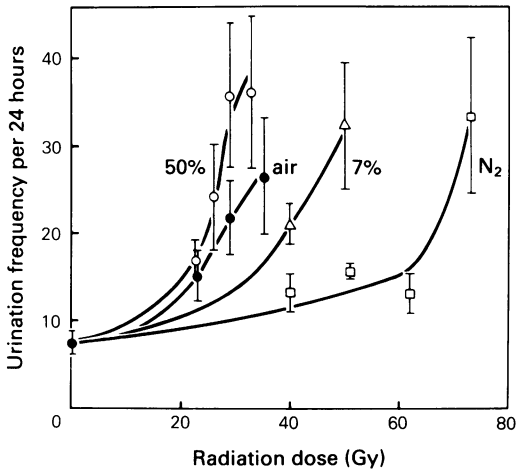

Figure 8 Dose response curves for urination frequency in mice at 9 months after electron irradiation in 50% O_2, air, 7% O_2, or N_2.

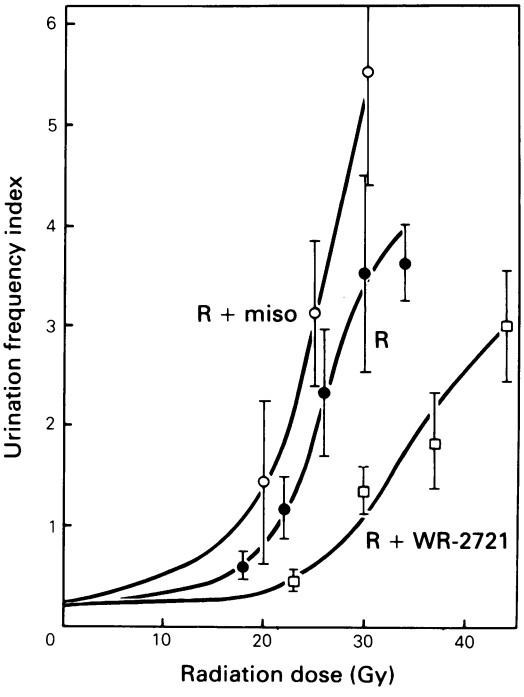

Figure 9 Dose response curves for urination frequency in mice at 12 months after irradiation in the presence of 1,000 mg kg^{-1} misonidazole or 400 mg kg^{-1} WR-2721. Frequency index (number of urinations in 24 h ml^{-1} of urine produced) is shown against electron dose.

epithelial necrosis which occurs during this late period favours the development of secondary infection, and calcareous deposits (formed in the presence of certain types of bacteria) are often found in the bladder lumen which further aggravates symptoms of increased urination frequency and dysuria (Watson *et al.*, 1947; Dean, 1933). Some of these clinical symptoms resolve when the bladder mucosa heals and it therefore seems likely that epithelial disturbances are responsible for the functional damage in such cases. A physical reduction in bladder volume capacity as the result of fibrosis has also been observed in some instances (e.g. Morrison & Deeley, 1965) and this condition obviously leads to *permanent* functional impairment.

In animal studies (Stewart *et al.*, 1978; Stewart *et al.*, 1980), the onset and development of increased urination frequency after irradiation correlates well with the presence of epithelial desquamation and compensatory regenerative proliferation. Calculi have also been found in up to 24% of irradiated mouse bladders at 14 months after treatment (Stewart *et al.*, 1986). Fibrotic bladders with a reduced volume capacity have, however, been observed in mice from 9 to 12 months, i.e. during the period in which urination frequency develops (Stewart *et al.*, 1981). Indeed, there appears to be a direct relationship between the extent of bladder contraction and urination frequency at times later than 12 months after irradiation. It is, therefore, still not clear to what extent epithelial disturbances, in particular the loss of superficial cells and exposure of non-specialized deeper layers to the hypertonic urine, contributes to urination frequency *before* the development of late radiation induced fibrosis.

In an effort to clarify this issue, an experiment was performed in which cyclophosphamide was given at 1 week after irradiation of the bladder (Stewart, 1985). The cyclophosphamide induced an immediate wave of cell death (beginning in the superficial, polyploid cells), with subsequent active proliferation. It was postulated that this proliferation would precipitate latent radiation damage which is not normally expressed until at least 6 months, but no such precipitation of damage was observed. Some increase in urination frequency occurred during the first few months after cyclophosphamide but this was entirely due to the drug alone and was not related to the radiation dose delivered. Radiation dose related increases in frequency were not observed until 7 months after treatment and occurred at the same time in groups of animals which had received radiation alone or radiation plus cyclophosphamide. These experiments demonstrate that epithelial damage is not necessarily involved in the late functional damage

which occurs after irradiation of the bladder. It had previously been suggested (Stewart *et al.*, 1980) that bladder fibrosis occured as a secondary result of epithelial denudation when deeper layers were exposed to the damaging effects of the urine. However, cyclophosphamide given after irradiation resulted in epithelial denudation and stimulated active proliferation but it did not produce early fibrosis or precipitate late radiation damage. It seems likely, therefore, that although epithelial ulceration and disturbance can cause functional damage (including increased permeability and frequency), this is probably not the primary event leading to late radiation damage.

Conclusions

The rate of cell turnover in the bladder epithelial cells is normally very slow but these cells can be stimulated into rapid proliferation by injury. The compensatory proliferative burst occurs rapidly (within days) after mechanical or chemical injury but after irradiation there is a delay of 6 months before the maximum response is observed.

Functional damage (increased urination frequency) occurs within 1 week of cyclophosphamide administration, immediately following epithelial desquamation. After irradiation no functional damage is seen until at least 6 months.

Cyclophosphamide given 1 week after irradiation does *not* precipitate latent radiation injury. Therefore epithelial denudation is probably not the primary cause of late radiation injury in the bladder.

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