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Feature Articles

Molecular Assays of Radiation-induced DNA Damage

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There is a need for assays of DNA damage in many areas of laboratory research applied to radiation therapy, in order to understand the molecular processes involved in cell killing by ionising radiation and to predict *in vivo* response. Assays exist which measure many types of DNA damage following ionising radiation. From studies of the dose-response relationships for different types of damage, the double-strand break (dsb) has been shown to be the most significant lesion. Assays for DNA dsb have been of low sensitivity, such that supralethal doses of radiation had to be used in order to study dsb induction or repair. New assays, such as pulsed-field gel electrophoresis, are sensitive to dsb in a dose range relevant to cell survival. In addition, these assays can assess the distribution of dsb in different parts of the genome and determine heterogeneity of damage induction and repair. Assays which measure the effects of strand breaks on DNA complexed with nuclear matrix can reveal features of chromatin organisation and their influence on cellular radiosensitivity.
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INTRODUCTION

THE LETHAL effects of ionising radiation are widely accepted to be caused by DNA damage. Measures of this damage are important for a number of reasons. Understanding the molecular processes underlying the response of cells to radiation may help in the search for agents to modify the response. This could benefit both radiotherapy by increasing tumour cell kill, and radioprotection by decreasing normal cell kill. In addition, rapid measures of the radiation response are required in both these areas. In radiotherapy the tumour response is, in part, determined by the inherent radiosensitivity of the tumour cells [1, 2]. There is a need to measure inherent radiosensitivity not just for prognostic information but also to define tumour and patient subgroups which may benefit from potential improvements in radiotherapy. Assays to assess the cellular radiosensitivity of an individual's tumour and normal tissues within a time scale, which makes them suitable as a predictive test have been largely unsuccessful. The development of a DNA damage assay which would predict the magnitude of cell kill is therefore attractive. Assays of DNA damage able to monitor environmental exposure (as well as determining an individual's sensitivity to that exposure) would be valuable in radioprotection.

A variety of lesions is produced by ionising radiation including single-strand or double-strand breaks (ssb or dsb), base and sugar damage, and crosslinks between DNA and proteins or other DNA strands. Unlike ultraviolet radiation or many cytotoxic drugs, no single DNA lesion has been identified following ionising radiation which leads irrevocably to cell death. Indeed it is likely that there is no single "lethal lesion" induced by ionising radiation. In the absence of a specific lesion, studies of the molecular processes underlying radiation-induced cell killing have been directed at correlations between damage and cell killing.

It is the DNA dsb which has received the most attention in recent years as the most significant lesion induced by ionising radiation. The evidence for this is based on a process of elimination, i.e. other lesions, such as DNA ssb or base damage, have been shown not to correlate with cell killing [3–5]. Also, agents which produce dsb specifically, e.g. restriction endonucleases, cause chromosome aberrations and cell killing in a similar way to ionising radiation [6, 7].

A considerable amount of work has been directed at providing sensitive and reliable assays of DNA dsb in irradiated cells. While much has been learned from measuring true DNA dsb, i.e. breaks in naked DNA, it is sometimes revealing to assess damage to DNA in a form closer to its cellular state, i.e. with components of nuclear protein. Some frequently used assays, which measure damage to DNA in both these forms, are outlined in Table 1. In this article we will focus on recent technical advances and discuss current ideas about the mechanisms of radiation response which are suggested by the use of these assays.

STRAND-BREAKAGE ASSAYS

The purest form of strand-breakage assay measures breaks in naked DNA (i.e. after removal of all cytoplasmic contents, organelles, membranes and nuclear proteins by detergent and proteolytic treatment). Such assays measure either changes in DNA mass using centrifugation, or changes in length by filtration or by electrophoretic mobility. Under neutral conditions, these assays are specific for dsb while under alkaline conditions (pH>11), the DNA double helix separates due to denaturation of interstrand bonds, which allows measurement of ssb. Several recent reviews have described technical aspects of the most

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Table 1. Principal features of the most important DNA damage assays

Assay	Lesion and dose range for determination	Principle	Advantages	Disadvantages	Ref.
Sucrose velocity sedimentation	ssb ≥ 5 Gy dsb ≥ 15 Gy	Larger DNA fragments sediment further in linear gradient	Absolute quantity of dsb	Insensitive to low doses Anomalous effects in cells with large genome	43
Filter elution	ssb 1 – 2 Gy dsb ≥ 5 Gy dpc ≥ 30 Gy base ≥ 2 Gy	Larger fragments elute more slowly	More sensitive than sedimentation	Uncertain effects of DNA conformation, cell cycle, cell number, lysis, pH	19, 54
PFGE	dsb ≥ 1 Gy	Mobility in gel determined by gel factors and DNA relaxation/reorientation rates	Sensitive at doses relevant to cell survival Heterogeneity of damage/repair can be measured	Uncertain effects of DNA conformation	24, 25, 26 27, 52
Alkaline GE	ssb ≥ 10 Gy	Fragment mobility depends on gel and fragment size	Simple, rapid	Effects of loading Conformation	55
Membrane filtration	dpc 10 – 100 Gy	DNA protein binds more strongly than naked DNA to filter	Applicable to circular vs. linear DNA crosslinks	Need to assess DNA fragment size to control for size effect on filtration	56
DNA precipitation	ssb 1 – 20 Gy dsb ≥ 40 Gy	Fragments have differential solubility	Rapid, simple Low cell numbers Large capacity	?Nature of lesion pH critical Result varies with type of treatment	57
Nucleoid sedimentation	ssb 1 – 20 Gy	Sedimentation of histone-free DNA depends on mass and degree of supercoiling in ethidium bromide	Sensitive, rapid Assesses DNA conformation as well as damage	Repaired nucleoids sediment less than controls Uncertain nature of lesion detected	30, 31
Alkaline unwinding	ssb 0.1 – 100 Gy	ssb indicated by ratio of ss/ds DNA after partial denaturation	Assesses topology of DNA conformation Base damage revealed with ess	Difficult to assess repair as kinetics of unwinding and rewinding vary	8, 37, 38
Halo	ssb 1 – 20 Gy	Nicked DNA loops unwind more in propidium iodide	Needs few cells Tumour cell-specific Rapid	Difficult to quantify Heterogeneity between cells	33, 34 36
Comet	ssb 0.5 – 3 Gy				
End tailing	ssb 2 – 50 Gy dsb ≥ 3 Gy	Site of strand break labelled by range of enzymes and radionuclides	Can discern chemical nature of strand break Also base damage by ess PCR can increase sensitivity	Poor reproducibility for dsb 2 enzyme steps for dsb Not yet applicable to mammalian cells	58, 59
Viscoelastic	ssb 0.3 – 10 Gy	Nicking of DNA loops causes unwinding and retards mechanical recoiling	Assesses conformation Sensitive at low doses	?Nature of lesion Saturation of response >10 Gy	60
HPLC	Base ≥ 20 Gy	Differential affinity for resin column	Rapid, specific	Insensitive	61, 62

Some detect strand-breakage in naked DNA while others assess the effects on chromatin conformation of strand-breaks. For a more thorough discussion of the techniques see text and references.

ssb = single-strand break, dsb = double-strand break, dpc = DNA-protein crosslinks, ess = endonuclease sensitive sites, GE = gel electrophoresis, PFGE = pulsed-field gel electrophoresis, PCR = polymerase chain reaction, HPLC = high performance liquid chromatography.

commonly used damage assays [e.g. 8–10] so here we will only briefly discuss three principal assays.

Sucrose velocity sedimentation

This is sometimes considered to be the "gold standard" of strand breakage assays. The underlying principle (Fig. 1a) is that DNA fragments sediment at a rate proportional to their size. Larger fragments sediment further in a linear sucrose gradient, that is of constant increase in concentration from top to bottom. Calibration with standards of known molecular weight allows the conversion of the distribution of DNA fragments in the gradient into a distribution of molecular weights. From this distribution the average fragment length can be calculated for a given dose, and hence a strand-breakage rate per unit length of DNA per Gy determined.

Direct quantification of the degree of fragmentation requires doses higher than 20 Gy. The low sensitivity of the assay is due to the high background of breaks from DNA shearing due to cell manipulation after lysis and anomalous movement of mammalian cell DNA. Thus, small changes in the efficiency of strand breakage at low doses (in the range relevant to cell killing) are not detectable.

Calculations from this assay have suggested that dsb are produced with a frequency of 5–10 $\times$ 10^{-9}/base pairs per Gy, which is equivalent to 30–60 dsb per diploid cell per Gy in mammalian cells [11, 12, 15]. In terms of resulting fragment size, dsb production appears random through the genome (indeed the calculations of dsb induction frequency assume this) and dsb induction is linear with dose.

Filter elution

This is a simpler, and more rapid assay (Fig. 1b). Cells are lysed directly on a filter with pores of 0.2 μm and buffer solution is pumped through the filter to elute DNA fragments. Fractions of eluate are collected over 16 hours and the level of strand breakage is indicated by the rate of DNA elution. The rate may depend on fragment size directly, with larger fragments taking longer to elute, but recent work [13] has found that eluted fragments are of a uniform size, around 460 kilobase pairs, (kbp), suggesting that a process of fragment disruption and further breakage occurs on the filter and it is this process which is proportional to the number of dsb induced.

This assay can detect dsb induced by doses of 5–10 Gy. No direct quantification of strand breakage efficiency can be obtained but the assay can be calibrated against a treatment which induces a known number of dsb. This is achieved by labelling cells with ^{125}I-iododeoxyuridine (which is incorporated into DNA), freezing the cells and assuming that each disintegration of ^{125}I causes on average 1 dsb [14, 15]. Such calibration has suggested that the elution rate is linearly related to the number of dsb.

Every effort has been made to characterise this technique but criticisms remain [16]. Cell lysis conditions are not as stringent as those used for sedimentation, so protein contamination may affect the measurement. For example, it has been suggested that variation in elution rate (when comparing different cell lines) may be due to variation in the DNA-protein complexes rather than to differences in dsb production [17]. Therefore, the interpretation of dose-response relationships for filter elution requires caution. The influence of strand breaks on such DNA-protein complexes may allow a measure of damage which is relevant for cell killing. The pH of the elution buffer is often raised from 7.4 to 9.6 to increase sensitivity, but this is thought

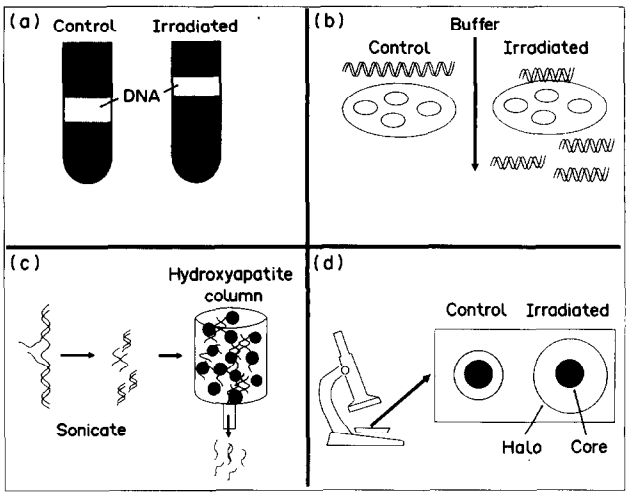

Fig. 1. Schematic representation of (a) sucrose velocity sedimentation, (b) neutral filter elution, (c) alkaline unwinding and (d) halo microfluorescence. For details of the techniques see text.

to allow the detection of a new set of lesions: those which are alkali labile and may not be expressed at cellular pH [16].

This technique has become the most widely used for measuring dsb. One finding which is common, though not universal, is that damage induction shows a curvilinear relationship with dose rather than linear as seen with sedimentation [18]. Attention has been focussed on the relative significance of the initial dsb induced and their repair as determinants of cell killing. Radford and colleagues have suggested that the level of damage detected in cells maintained on ice, i.e. in the absence of enzymatic repair, correlates well with cell killing [19]. However, other authors have reported defects in dsb rejoining in radiosensitive cells with similar levels of dsb induced, suggesting that repair is the determining factor [20–23].

Pulsed-field gel electrophoresis (PFGE)

This is the newest strand-breakage assay and is likely to replace filter elution in the measurement of DNA dsb. Since its initial description in 1984 [24] it has been developed as an aid to gene mapping by allowing resolution of larger DNA fragments produced from restriction endonuclease digestions. More recently PFGE has been applied to measure DNA strand breakage [25–28]. When DNA is forced to move through an agarose polymer gel under a unidirectional, constant strength electric field, molecules under 30 kbp migrate at a velocity proportional to their size but molecules over 30 kbp move at a constant velocity. Hence large fragments of DNA cannot be separated by size. Alternation of field direction or strength is termed field "pulsing" and this forces large molecules to relax and reorientate before recommencing migration in the new field direction. This process is size-dependent and allows resolution of large DNA molecules (Fig. 2). Resolution is facilitated by using an optimum angle between the fields and long pulse times (over 1 hour) at low field strength (less than 5 volts/cm). Under these conditions fragments up to 12 Mbp can be separated.

Dsb induction is quantified by the fraction and distribution of DNA migrating into the gel. Partly because of the gentle physical treatment of the cells, this technique is sensitive to doses as low as 1 Gy. Using molecular weight markers derived from yeast chromosomes, the fragment size distribution can be measured and the dsb induction efficiency determined. We have found values of around 3–5 $\times$ 10^{-9} dsb/bp/Gy in human tumour

cells (S.J.W. and T.J.M.), which is comparable with the values found with sedimentation [11].

Using PFGE, Ahn *et al.* [29], have recently measured dsb rejoining in mammalian cells irradiated with doses as low as 5 Gy. These experiments are of particular importance as other strand breakage assays require supralethal doses of X-irradiation to follow dsb rejoining. The dsb rejoining measured by these assays derives predominantly from cells destined to die. At doses below 5 Gy, however, there is a higher probability that a cell will survive and thus dsb are more likely to reflect significant lesions for cell killing.

ASSAYS THAT ASSESS DNA HIGHER ORDER STRUCTURE

In order to accommodate a molecule of DNA, which is 50–100 cm in length within a nucleus of 5–10 μm diameter, the DNA has to be condensed by a factor of 10^5 and yet it has to remain accessible for replication, transcription and repair. This packing is achieved by the formation of a higher order structure of DNA with proteins.

DNA can be isolated whilst retaining some of this structure. Following detergent cell lysis and high salt denaturation (1–2 mol/l NaCl) but without proteinase, DNA is in a supercoiled form called a "nucleoid" which is free of histones but retains some of the higher organisation of chromatin due to other associated proteins of the nuclear matrix [30]. The degree of winding of these nucleoids can be modified by the fluorescent dye ethidium bromide. This dye intercalates between DNA bases and, at increasing concentration, progressively unwinds the normally negative supercoiling. Further increase in dye concentration then induces supercoiling in the opposite or positive sense.

Nucleoid sedimentation

In this assay the degree of supercoiling modifies the sedimentation of a nucleoid in a sucrose column by altering its density. Further changes in this density are imposed by radiation since the presence of strand breaks relaxes the DNA. Thus a relationship can be seen between the radiation dose and the sedimentation distance of the nucleoids in a given concentration of ethidium bromide [30].

Analysis of nucleoids has led to some interesting clinical correlations. Deeley and Moore [31] demonstrated decreased repair in a nuclear lysate sedimentation assay in lymphocytes from patients with bowel complications due to radiotherapy. Vaughan *et al.* [32] have reported a correlation between nucleoid sedimentation in tumour cells present in urine and tumour response in patients with carcinoma of the bladder.

Halo microfluorescence

Microscopy allows detection of fluorescence from single cells and a measurement of the degree of chromatin loop unwinding which is independent of the mass of the nucleoid [33, 34]. This is performed on nucleoids stained with propidium iodide (PI; see Fig. 1d). In unirradiated cells exposed to a low concentration of PI the unwound DNA loops are seen as a "halo". With increasing concentration of PI the loop size, and thus the halo, increases to a maximum diameter, then reduces due to positive supercoiling or "rewinding". Following irradiation two changes are observed. Firstly, the loops unwind to a greater degree for a given concentration of dye (the maximum halo size increases). Second, the loops do not completely rewind (unless repair of strand breakage is allowed). Using this technique Taylor *et al.* [35] have shown abnormalities in the halo formation of diploid fibroblasts from patients with ataxia-telangiectasia (AT) compared with those from normal individuals. They found an increase in the maximum halo size and reduced rewinding after irradiation and have speculated how these differences may relate to the radiosensitivity of the AT cells.

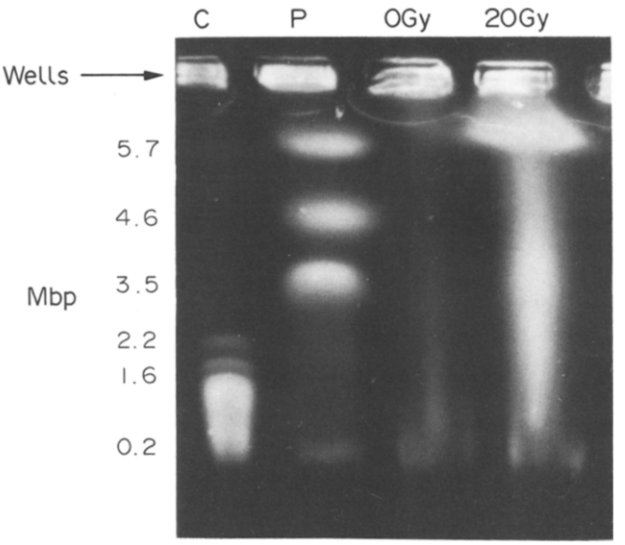

Fig. 2. Typical gel following pulsed-field gel electrophoresis of irradiated human tumour cell DNA. Lane 1 (C) carries *Saccharomyces cerevisiae* chromosomes (only the largest 2 of 15 chromosomes are seen, of 2.2 and 1.6 Mbp), and lane 2 (P) carries *Schizosaccharomyces pombe* chromosomes of 3.5, 4.6 and 5.7 Mbp as molecular weight markers. Lanes 3 and 4 show the typical DNA smears from a bladder carcinoma cell line. Lane 3 carries unirradiated controls while cells in lane 4 have been irradiated on ice with ^{60}Co gamma-rays with 20 Gy. PFGE parameters: Clamped homogeneous electric field (Bio Rad CHEF-DRII) system, 60 minute switching (2 fields ±60° to net DNA migration), 4 volts/cm, for 96 hours. Gel = 0.8% agarose, buffer = 0.5 × TBE at 16°C.

Comet assay

This is a similar technique to the halo assay which involves embedding the cells in agarose gel. Following lysis the nucleoids are subjected to a brief electrophoretic separation where the relaxed coils of chromatin spread out from the nucleoid towards the anode to form a "comet" [36]. The size of the tail of the comet is the endpoint used.

Alkaline unwinding

This assay allows partial unwinding of double-stranded DNA under brief alkaline/high salt denaturation [8, 37, 38] (see Fig. 1c). Reannealing of single strands is prevented by a brief ultrasonic shock which randomly fractures all the DNA into small fragments. The resulting proportion of double-stranded to single-stranded DNA fragments produced is measured by differential binding to and then elution from a hydroxyapatite column. The ratio of single-stranded to double-stranded DNA reflects the initial induction of ssb. However, this ratio may be influenced by the higher order structure of DNA when irradiated due to physical "constraints" to unwinding rather than altered strand breakage. One model proposed from these observations involves the nuclear matrix attachment sites. These attachments are believed to be the sites of DNA replication, transcription and possibly repair [39]. It is speculated that increased constraints correlate with increased levels of poly-(ADP-ribose) polymerase

in these cells [40]. An alternative finding is of increased retention of a 55–60 kD protein in nucleoids demonstrating more DNA constraints and it is speculated that this protein maintains the structural integrity of DNA and may influence repair [41].

Rather than simply measuring strand breakage, these assays provide information on structure which may influence the radiation response in cells. The data obtained with these techniques is still sparse, however, and there is no clear picture as to its significance to cell killing by ionising radiation. At its extreme this is demonstrated by two comparisons: V79 cells as spheroids have more structural constraints and are more radioresistant than V79 cells as monolayer cultures [41, 42] while in contrast Ewings sarcoma cell lines are more sensitive than HeLa but are also more constrained [40].

THE MOLECULAR BASIS OF RADIOSENSITIVITY

The work of Radford using neutral filter elution has challenged some established ideas in radiobiology. Sucrose sedimentation assays suggested that damage is induced linearly with dose [43]. Radford suggests that this relationship is curvilinear and since the physical deposition of energy must increase linearly with dose then non-enzymatic chemical reactions are probably influencing the amount of damage detected in the DNA [19]. This claim is supported by experiments where scavengers of radiation-induced free radicals (such as cysteamine) and modifiers (such as oxygen) change the level of induced damage to the same degree as they modify cell kill. That differences in radiosensitivity between cell lines may also be due to changes in initial damage [4, 19] has been less easy for radiobiologists to accept. From radiochemistry studies, Ward [44] suggests that the levels of natural chemical modifiers are unlikely to be present in sufficient concentrations in the vicinity of the DNA to effect radical scavenging. However, he does suggest that differences in chromatin conformation may be a mechanism by which initial damage is altered.

Differences between cell lines in dsb rejoining have been detected infrequently. Some radiosensitive mutants of rodent cell lines do appear to have a defect in rejoining [20–22] but in the majority of cases normal rejoining has been found. Even in the highly radiosensitive syndrome, AT, there has only been one case report of a defect in dsb rejoining [45]. In the case of other AT cells the defect has been suggested to be associated with fidelity of repair rather than extent of rejoining and indeed that may be the case for several other radiosensitive cells (ref. 46 and S.N.P., unpublished).

How important is it to measure the lethal lesion specifically? For damage induction studies some agents (e.g. cysteamine) can protect against most types of damage; therefore identification of the lethal lesion is not critical [5]. In comparing between cell lines, however, there is evidence that the proportions of different lesions produced varies, but we would expect changes in the number of dsb to parallel changes in the level of lethal lesions [5]. Also, modifiers such as hyperthermia are more specific in the type of damage they influence and more care has to be taken in interpreting results. With repair studies the situation is different since different lesions vary in their repairability; so their relative contribution to the final level of lethal damage varies. Even with dsb the majority of lesions disappear after a few hours and only a minority persist. It is attractive to imagine that the final level of dsb determines cell killing, but evidence for this is sparse. To date, the low sensitivity of existing damage assays cannot resolve whether small changes in residual dsb determine cell survival or death.

THE FUTURE FOR DAMAGE ASSAYS

PFGE appears to have the greatest potential to improve the sensitivity and specificity of DNA dsb measurement. It is already almost an order of magnitude more sensitive than other techniques. PFGE also has the scope to be extended to more detailed studies of the nature and distribution of radiation-induced damage. Southern blotting of the gels, hybridised to specific genetic probes, for example, opens up the opportunity to examine whether damage induction or repair is random or variable throughout the genome. Previous studies have found damage induction and repair to be distributed non-uniformly through the genome with more ssb, dpc and base damage induced in actively transcribing regions and in newly replicated chromatin [47–51]. Field-inversion gel electrophoresis has been applied to dsb measurement at pH 9.5 and no difference was found in damage in an amplified oncogene (c-*myc*) compared to an inactive gene (globin) [52]. These were early experiments and the increased sensitivity of the latest PFGE systems should explore this further.

The sensitivity of PFGE should also help in the examination of those lesions which persist after a significant repair time. It has been found that dsb with different structures, (i.e. blunt or staggered ends), differ in their biological effectiveness [6, 7, 53]. This raises the possibility that residual dsb, rather than being random, may represent the persistence of a particular subset of dsb which are more difficult to repair or are even irreparable. An important aim will be to resolve whether such lesions are produced at the time of irradiation or are a consequence of error-prone repair processes.

How useful might these assays be in the clinical setting? As a rapid test of the extent and mechanism of radiosensitivity in human tumour biopsy material, the sensitivity of strand-breakage assays such as PFGE is now at a level to answer the question of how the number of residual dsb correlates with sensitivity. Existing clonogenic assays of cellular radiosensitivity measure the surviving fraction at 2 Gy, but these involve tumour cell culture. This is slow and may lack tumour cell specificity. Assays which measure the effect of strand breaks on DNA conformation are rapid and sensitive and can be applied to low numbers of cells. In addition, microcell assays, such as the comet or halo assays, assess DNA damage in single cells and may overcome the problem of tumour or normal cell specificity.

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Cancer Incidence in New South Wales, Australia

Margaret McCredie, Marylon Coates, Tim Churches and Richard Taylor

In 1972, cancer registration began in New South Wales (NSW), the most populous state in Australia. The operations of the Registry are described. By 1990, approximately 316 000 new cases of cancer had been notified from a population that had increased from 4.6 to 5.8 million. In 1981–1984, the most common sites in men were lung, prostate, colon, melanoma and bladder, and in women, breast, melanoma, colon, lung and unknown primary site. Cancers which, between 1973–1976 and 1981–1984, had increased in reported incidence by more than 25% were pharynx and kidney in both sexes, rectum, testis and melanoma in men, and lung and bladder in women; those decreasing by more than 10% were stomach in both sexes, oesophagus in men and cervix in women. Age-standardised incidence rates for melanoma (27.4 [m] and 23.8 [f] per 100 000 in 1987) and cancer of the renal pelvis in women (1.7 per 100 000 in 1989) are among the highest in the world.
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INTRODUCTION

NEW SOUTH WALES (NSW) is the most populous of the six federated states of Australia. In 1971 the NSW Central Cancer Registry was established to collect statistics for the state from January 1972. NSW, a temperate zone between 28° and 38° south and 141° and 154° east, has an area of 801 400 square kilometres excluding the Australian Capital Territory which is bordered on all sides by NSW. The population, in millions, was 4.6 in 1972, 5.4 in 1984 and 5.8 in 1990. In 1984, 31% of the population was aged less than 20 years and 11% 65 years or over. A coastal population predominates, 76% living in the Sydney–Newcastle–Wollongong conurbation along the central coast. At the 1986 census, 89% of the population lived in urban areas and 22% were born overseas.

This report presents a description of the operation of the Registry, and for the period 1972–1984, a summary of cancer incidence in NSW, indicating the most common cancers and those sites which have changed most over time. More recent data are presented for melanoma and cancer of the renal pelvis, sites which are particularly common in New South Wales.

OPERATION OF THE REGISTRY

Type of data

All new cases of cancer in residents of NSW are notified to the Registry, exceptions being basal and squamous cell carcinomas of the skin and *in situ* cancers at any site. Information is collected relating to identifying and demographic characteristics, and to the diagnosis of cancer but not to risk factors for cancer, such as occupation or smoking habits.

In the period 1972–1984, most cases were verified histologically (average for all cancers combined was between 87% and 92%) and less than 1% of cases were reported by death certificate only.

Sources of data

The main source of data is the compulsory notification form completed by medical or records staff for each admission of a patient with cancer to every hospital, both public and private, and nursing home. In addition, each radiotherapy department must notify the first attendance each year of each cancer case. Other sources of data are pathology reports relating to cancer received from all major hospitals and private pathology laboratories, voluntarily up to 1985 and then as a legal requirement. Since 1972, the Registrar-General's Department has supplied listings of all deaths certified as having cancer as the primary cause, with supplementary listings of cancer as the secondary cause from 1978. Tapes of death from all causes have been supplied from 1985, and direct computer matching will be carried out against Registry files. In 1990, there are approximately 308 000 persons registered representing 316 000 new cases of cancer.

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