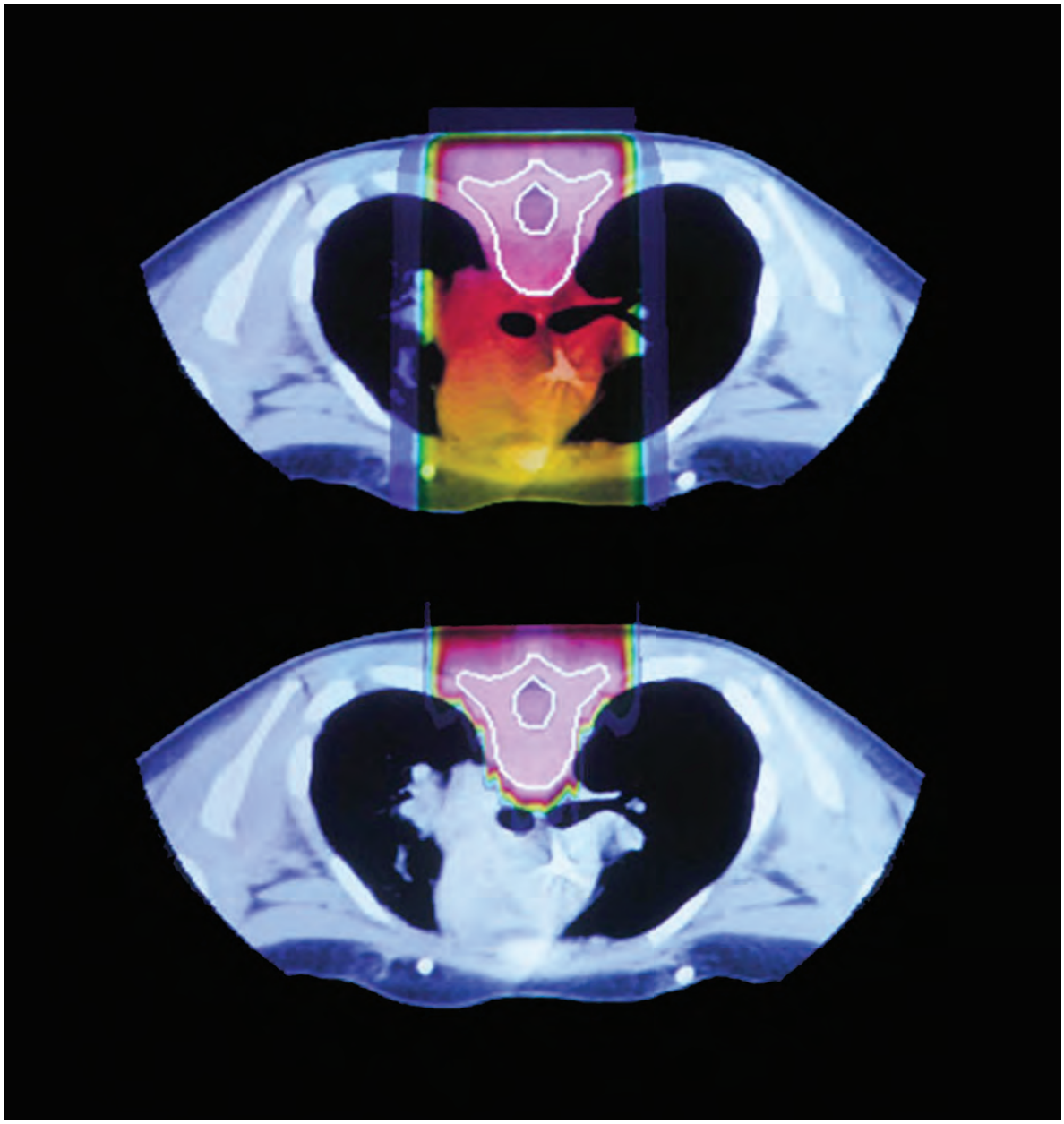
Health Protection Agency

Circulatory Disease Risk

Report of the independent Advisory Group on Ionising Radiation



The cover shows medulloblastoma spinal treatment plans using either X-rays (top) or protons (bottom), as used at the Massachusetts General Hospital. The extent of dose transmission through the body for X-rays compared to none for protons can clearly be seen.

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Report of the independent Advisory Group on Ionising Radiation

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Foreword

The Health Protection Agency (HPA) has a statutory responsibility for advising UK government departments on health effects and standards of protection for exposure to ionising and non-ionising radiations. This responsibility came to the HPA in April 2005 when it incorporated the National Radiological Protection Board (NRPB).

In 1995 the Director of the NRPB set up the Advisory Group on Ionising Radiation (AGIR) that had as its terms of reference:

> 'to review work on the biological and medical effects of ionising radiation relevant to human health in the occupational, public health, medical and environmental fields and advise on research priorities'

In addition, the AGIR was given the task of helping the then NRPB, where appropriate, to deal with any urgent request for advice or work from the Department of Health or other government departments. The AGIR was reconstituted in 1999 as an independent body and reported directly to the Board of the NRPB; it now reports to the Board of the HPA. The remit of the AGIR is restricted to the provision of scientific judgements and does not include the development of specific recommendations relating to radiation protection policy. These are matters for the HPA and its Board. For details of the current work of AGIR, see the website at www.hpa.org.uk.

The AGIR has, to date, issued seven reports that consider:

a heterogeneity in response to radiation,

b guidance on promotion of further optimisation of medical exposures,

c epidemiology of second cancers,

d UK population risks for leukaemia,

e review of risks from tritium,

f high dose radiation effects and tissue injury,

g radon and public health.

This report is based on work conducted by the AGIR Subgroup on Circulatory Disease Risk. The report reviews the epidemiological, experimental and medical evidence for radiation exposure elevating the incidence of circulatory diseases. Increased evidence of an association between circulatory diseases and radiation exposures has become available over recent years. Radiological protection does not currently include circulatory diseases in the calculation of health detriment from low dose exposures. It is therefore timely to review the evidence for radiation association and causation of circulatory diseases and consider the likely need and implications for radiological protection to take these diseases into account in protection of health from low dose exposures and medical exposures.

Circulatory Disease Risk

HAS BEEN PREPARED BY THE

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Circulatory Disease Risk

Report prepared by the Subgroup on Circulatory Disease Risk of the independent Advisory Group on Ionising Radiation

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This report from the independent Advisory Group on Ionising Radiation reflects understanding and evaluation of the current scientific evidence as presented and referenced in this document.

1 Introduction

Circulatory diseases are common in Western populations and are the main cause of death in the UK, accounting for some 193,000, or 34%, of deaths each year (British Heart Foundation, 2010). Circulatory diseases are those that affect the heart or blood vessels and include the atherosclerotic diseases of stroke and coronary heart disease, pericardial disease and myocardial dysfunction amongst others (see Box 1). Coronary heart disease is the most common cause of death in the UK, with 94,000 attributable deaths per year, affecting about one in five men and one in seven women. Stroke is the third most common cause of death, leading to 53,000 deaths per year in the UK, after cancer – the second most common cause. Multiple contributary risk factors for circulatory disease have been identified, including potentially controllable dietary factors and lifestyle factors, metabolic factors that may be controlled such as hypertension and high blood cholesterol levels, and fixed factors such as gender, genetic predisposition and age. Therefore there are multiple contributory factors that may modify circulatory disesase risk in humans.

Environmental agents may also contribute to circulatory disease risk and it has long been recognised that human exposure to ionising radiation during radiotherapy can damage the heart and vessels such as the carotid and coronary arteries. This has been confirmed in experimental animal studies (see Adams et al, 2003, for a review). Radiotherapy doses to the heart can be very high as in the treatment of Hodgkin's disease where doses to some regions of the heart can exceed 40 Gy (McGale and Darby, 2005). More recently there have been indications from radiation epidemiological studies of elevated circulatory disease risk at substantially lower doses. In particular, the Life Span Study of the survivors of the atomic bombings in Japan provides evidence of increased risk of myocardial infarction and stroke at lower doses (eg Yamada et al, 2004; Shimizu et al, 2010). The atomic-bomb survivor data are further compatible with a linear no-threshold radiation dose–response, although the actual form of the dose–response relationship is uncertain. Therefore the magnitude of the risk of circulatory disease in the low dose region where issues of radiation protection usually operate is not clear. Epidemiological studies are likely to have difficulty in detecting increased risk at low dose levels as the main circulatory diseases of concern are very common in the population as a whole and there are multiple potentially confounding contributory risk factors. Experimental animal studies provide evidence that all structures of the heart and coronary arteries can be damaged by ionising radiation exposure and animal model systems have been developed to study particular disease-related endpoints. The radiobiology of cardiovascular disease has been reviewed previously (Schultz-Hector, 1992; Schultz-Hector and Trott, 2007; Hendry et al, 2008). Several reviews on the effects of radiation on circulatory disease have been published recently that pay particular attention to the possible effects of lower doses (eg Little et al, 2008; Darby et al, 2010).

Currently radiation protection standards aim to protect individuals from deterministic effects completely and minimise the risk of stochastically induced disease. Therapeutic medical exposures are explicitly excluded as the judgement of clinical benefit weighed against harm is best made on an individual basis by

BOX 1 Types of Circulatory Disease

There are multiple types of circulatory disease and the list below is not exhaustive. Damage to the vasculature can affect the function of most body organs through restriction of blood flow and oxygen to tissue; however, it is mainly the heart and brain that are of concern. Only certain circulatory diseases are considered to be affected by radiation exposure – these appear in *italics* in the list below.

Congenital heart disease

Includes a range of abnormalities in heart structure or function that are present at birth. Such conditions could potentially be caused by irradiation of the fetus but obstetric irradiation is carefully controlled (see HPA et al, 2009).

Cardiac valve diseases

Include a variety of abnormalities to the heart valves such as mitral stenosis and tricuspid regurgitation.

Hypertrophic cardiomyopathy

Increased muscle density in the heart leading to less effective pumping of the blood.

Cardiac arrythmias

Abnormally slow (brachycardia) or fast (tachycardia) beating of the heart often attributable to abnormalities in the electrical signalling that coordinates the beating of the four chambers of the heart.

Pericarditis

Inflammation of the pericardium, the membrane that surrounds the heart, most frequently attributable to infectious agents but also well established to be caused by high doses of radiation.

Coronary heart disease

Obstruction of the blood flow in the heart due to narrowing of cardiac vessels restricting blood and oxygen supply to the heart. In a mild form this leads to *angina* where the reduced blood flow results in discomfort. When blockage is severe, *myocardial infarction (heart attack)* occurs leading to acute heart failure.

Congestive heart disease

This results from damage to, or reduction in, capacity of the heart muscles, leading to reduced and at times insufficient cardiac output.

Stroke

Interruption of the blood supply to the brain due to blockage or rupture of vessels – loss of blood and oxygen to areas can lead to cell death and consequently permenant brain dysfunction. Three major forms of stroke are recognised, ischaemic stroke caused by blockage due to blood clots forming locally *(thrombotic stroke),* bleeding in the brain as a result of a ruptured artery *(haemorrhagic stroke),* or fragments from distant clots lodging in the brain vasculature *(embolic stroke).*

clinicians (ICRP, 2007). Radiation protection is generally concerned with doses of 100 mSv or less and the risk of circulatory disease is not included in the calculation of radiation detriment from stochastically induced diseases (ICRP, 2007). This decision is based primarily on the sparse evidence of increased risk at doses below 500 mSv (UNSCEAR, 2006; ICRP, 2007). The ICRP is undertaking a review of non-cancer diseases associated with radiation exposure (ICRP, 2009) and this could result in changes to the ICRP position on circulatory disease.

People are exposed to radiation in a variety of contexts ranging from radiotherapeutic to environmental exposures and it is important that there is reliable and accurate information available on the magnitude of risk at all dose levels to inform health protection decisions. The terms 'high', 'medium' and 'low' are frequently used to qualify and specify radiation dose levels but the meaning of these terms differs substantially depending on the context. For example, 'low' to a radiotherapist might be doses less than 5 Gy, while to a radiation protection specialist, 'low' would be restricted to doses below 100 mSv. For clarity, throughout this report the following definitions will apply:

Very high – doses above 15 Gy

High – doses of 5–15 Gy

Medium – doses of 0.5–5 Gy

Low – doses below 0.5 Gy

The relationships between radiation parameters, absorbed dose, quality/weighting factors and monitoring, protection and risks, are shown in Figure 1.1. For protection purposes, the evaluation of risk for tissue reactions (deterministic effects) is quantified by ICRP convention as per unit dose (gray, Gy, or RBE-weighted Gy) (Figure 1.1). The evaluation of risk for stochastic effects, such as radiation-induced cancer or hereditary effects, employs a system involving radiation and tissue weighting factors to convert the doses in gray to an equivalent dose to a tissue or organ, and also to an effective dose to the whole body.

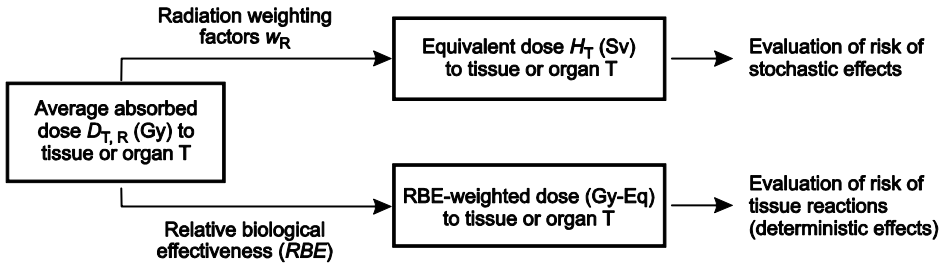


FIGURE 1.1 Use of radiation weighting factors and sieverts (Sv) for stochastic effects, and RBE-weighted doses (Gy-Eq) for tissue reactions (deterministic effects), in the ICRP system of quantities for radiation protection purposes (For further details see ICRP, 2007: Annex B. Figure supplied by Professor Jolyon Hendry)

As discussed in this report, the mechanism of causation of cardiovascular disease in the context of this conventional framework is not understood. As radiation-induced cardiovascular disease cannot be assumed to be stochastic in nature, whatever its mechanism, throughout this report preference is given to quoting radiation exposures in terms of absorbed dose measured in gray or as RBE-weighted Gy where the RBE is specified. However, in the literature, the sievert (Sv) or mSv has been used extensively. This is because in some cases the studies have arisen from investigations that were designed originally to assess cancer induction, or subsequent studies have used the same dosimetric quantities as previously for comparison and consistency. This practice has been continued for many years in radiation-related professions without regard to its validity, until recently when stochastic mechanisms are being questioned because of effects being detected at quite low doses. In some cases the original values in gray are not available. For this reason, the present report contains several tables using sievert rather than gray, and this caveat regarding the terminology is recognised and discussed.

The aim of this report is to provide information on the magnitude of risk of circulatory disease at all radiation dose levels and a consideration of the possible mechanisms by which radiation elevates circulatory disease risk and, based on these, a consideration of the risk projection models that might apply and the implications of such models for low dose radiation protection.

2 Recognised Contributory Risk Factors for Circulatory Disease

2.1 Cardiovascular Disease

As discussed in Chapter 1, the term circulatory disease comprises many conditions that vary widely in terms of their manifestations, aetiology and public health impact. Cardiovascular disease includes coronary heart disease (CHD), stroke and peripheral arterial disease, and is the most common cause of death and disability worldwide. The underlying cause of coronary heart disease, stroke and peripheral arterial disease is, in the vast majority of cases, atherosclerosis.

Amongst the main determinants of cardiovascular disease are age, sex and heredity. All three are considered important non-modifiable risk factors for cardiovascular disease. The risk of cardiovascular disease rises steeply with age; it is estimated that over 83% of people who die of CHD are 65 years or older (American Heart Association, AHA, 2010a). The association of CHD with age reflects the progressive accumulation of atherosclerosis in the arteries, which in turn reflects the cumulative exposure to atherosclerotic risk factors (National Cholesterol Education Program, NCEP, 2002). The rise in absolute risk with ageing becomes most clinically significant in men in their mid-forties and in women about the time of the menopause (NCEP, 2002). At any given age, men are at greater risk for cardiovascular disease than are women; the risk of death due to CHD in women is roughly similar to that of men ten years younger. Finally, cardiovascular disease has been shown to run in families and children of parents with cardiovascular disease are more likely to develop it themselves. The relative risk for CHD in first-degree relatives ranges from two to twelve times higher than that of the general population (Slack, 1969; Rissanen et al, 1979). Advances in genetic epidemiology over the past few years have helped to identify several genetic polymorphisms that increase or decrease an individual's chance of developing cardiovascular disease (Ioannidis, 2009). However, such genetic polymorphisms have so far been associated with small effects on cardiovascular risk.

Blood lipids levels have been repeatedly and consistently associated with cardiovascular disease. Increased serum cholesterol is and has been the *sine qua non* for experimental production of atherosclerosis in laboratory animals, including non-human primates (Stamler and Neaton, 2008). Serum cholesterol has been shown consistently to be a significant risk factor for CHD and other major cardiovascular diseases. The relationship is continuous, graded, strong, independent of other risk factors, predictive and generally assessed as aetiologically significant (Stamler et al, 2000). A large meta-analysis of 61 prospective studies has shown that 1 mmol L^{-1} lower total cholesterol was associated with about a half (hazard ratio* 0.44), a third (0.66) and a sixth (0.83) lower CHD mortality in both sexes at ages 40–49, 50–69 and 70–89 years, respectively (Prospective Studies Collaboration, PSC, 2007). However, associations between serum

* The hazard ratio is the ratio of risk in one group compared to another. In this case it is for the group with 1 mmol L^{-1} lower total cholesterol compared to a control group without such reduced cholesterol levels.

cholesterol and stroke are less clear, with most studies failing to show an independent association between cholesterol levels and ischaemic stroke (PSC, 2007). Besides total serum cholesterol, individual lipoproteins have shown associations with atherosclerotic disease. The Framingham Heart Study, the Multiple Risk Factor Intervention Trial and the Lipid Research Clinics trials found a direct relationship between levels of low density lipoprotein (LDL) cholesterol and the rate of CHD in men and women who were initially free of CHD (NCEP, 2002). These findings have been replicated by numerous population studies. Strong epidemiological evidence also links low levels of serum high density lipoprotein (HDL) cholesterol to increased CHD morbidity and mortality (Abbott et al, 1988; Wilson et al, 1998). A 1 mg dL^{-1} increase in HDL cholesterol has been associated with 2% and 3% increased CHD risk in men and women, respectively (Gordon et al, 1989). In addition to observational evidence, both clinical trials and angiographic studies have shown that reductions of serum total or LDL cholesterol levels are associated with reduced cardiovascular disease risk, and reduced atherosclerosis burden or progression.

Blood pressure is another well-established, strong risk factor associated with cardiovascular morbidity and mortality. Globally, approximately two-thirds of stroke and one-half of ischaemic heart disease are attributable to non-optimal blood pressure (Lawes et al, 2006). The Prospective Studies Collaboration showed that there is no threshold level of blood pressure, at least down to 115/75 mm Hg (within the range commonly occurring in Western populations) (PSC, 2002). In six populations in different parts of the world, the relative risk of death due to CHD was 1.17 per 10 mm Hg increase in systolic pressure and 1.13 per 5 mm Hg increase in diastolic pressure (van den Hoogen et al, 2000). The effects of blood pressure on ischaemic and haemorrhagic stroke mortality have been shown to be similar. Clinical trials have established that blood pressure reduction in people with hypertension reduces risk for a variety of blood-pressure-related endpoints including CHD (NCEP, 2002).

Extensive evidence links smoking to increased risk of cardiovascular disease development and progression; approximately 30% of heart-related deaths worldwide can be attributed to smoking (AHA, 2010a). The relationship of smoking with cardiovascular disease risk is both dose- and duration-dependent and observed in both men and women. The landmark study of British doctors reported significant trends with respect to smoking status and the amount of smoking with 13 categories of vascular mortality (Doll et al, 2004). The international study INTERHEART showed that the odds of developing myocardial infarction (MI) were increased by 1.06 for every additional cigarette smoked per day. The odds were 9-fold higher in those who smoked 40 or more cigarettes a day than in never-smokers (Teo et al, 2006). The risk of MI has been shown to fall progressively with time after smoking cessation but even in people who have quit 20 or more years ago, there is a residual excess risk of about 22% (Doll et al, 2004).

Diabetes is defined as fasting blood glucose of 126 mg dL^{-1} or greater. The risk for all forms of cardiovascular disease is increased substantially with the presence of type 1 or type 2 diabetes mellitus, and patients with diabetes who develop cardiovascular disease have a worse survival rate than do cardiovascular disease patients without diabetes (NCEP, 2002). A prospective analysis of 121,046 women aged 30 to 55 years with type 2 diabetes showed that the risk of CHD mortality increased monotonically with increased duration of the disease (Hu et al, 2001). The age-adjusted relative risks of fatal CHD increased from 2.8 to 11.9 in women with diabetes for five or fewer years compared to those with diabetes for more than 25 years, respectively. However, improved glucose control in diabetes patients has not been consistently shown to reduce incidence of cardiovascular disease (Tzoulaki et al, 2009).

An atherogenic diet is a major, modifiable risk factor for cardiovascular diseases. Diets that are high in caloric density, total fat, cholesterol, and saturated and transfats; high in salt and processed sugars; for some, excessive in alcohol intake; and relatively inadequate or low in key micronutrients and macronutrients from vegetables, fruits, whole grains and legumes – eg calcium, iron, magnesium, phosphorus, fibre and vegetable protein – have all been associated with major cardiovascular risk factors such as increased serum cholesterol levels, blood pressure and diabetes, and with the development and progression of cardiovascular diseases (Stamler and Neaton, 2008).

Physical inactivity and obesity are also associated with an increased risk of cardiovascular disease. In the Nurses' Health Study, three hours of brisk walking per week were associated with a 30–40% lower rate of myocardial infarction than that in sedentary women (Manson et al, 1999). Similarly, the Health Professionals Follow-up Study reported that men who walked briskly for at least 30 minutes per day were 18% less likely to develop CHD. The risk for cardiovascular disease is also particularly raised when abdominal obesity is high (Pischon et al, 2008). However, much of the risk associated with being overweight and obesity appears to be mediated through the major risk factors described above, as obesity is associated with all cardiovascular risk factors and thus its independent role on cardiovascular disease is not yet established.

Novel or emerging risk factors and biomarkers for cardiovascular disease are continuously being proposed (Tzoulaki et al, 2010). Many of these factors have shown consistent associations with cardiovascular disease, such as high sensitivity C-reactive protein levels, and may represent biomarkers of disease extent or activity (Shah et al, 2009). However, in the absence of clinical trial data that associate direct treatment of these biomarkers with reduction of cardiovascular disease rate, these proteins are still not considered conventional cardiovascular risk factors. Furthermore, as new biomarkers are often highly correlated with established risk factors, their additive predictive value over and above conventional risk factors has been questioned. Novel risk factors include several inflammatory and haemostatic biomarkers, lipoprotein particles other than LDL and HDL, lipoprotein remnants, homocysteine and others (Helfand et al, 2009). Finally, the presence of subclinical atherosclerotic disease is considered a strong risk factor for the development of clinical events (Ankle Brachial Index Collaboration, 2008). Subclinical disease can be measured by several non-invasive tools and may be considered as an emerging risk factor for cardiovascular disease.

2.2 Other Circulatory Diseases

Strong contributory risk factors have only been identified for atherosclerotic disease and it is primarily atherosclerotic disease that is of concern following radiation exposure at low and moderate doses; however, for completeness information is given below on the potential risk factors for some other classes of circulatory disease.

2.2.1 Valvular heart disease

Valvular heart disease involves one or more of the valves of the heart and may be congenital or acquired. Most common forms of the disease are aortic or mitral stenosis. The main causes of acquired valvular stenosis include rheumatic heart disease and degenerative calcification. The major risk factors for calcific

valve stenosis include the aforementioned risk factors for atherosclerotic disease such as age, gender, high total or LDL cholesterol levels, low HDL cholesterol levels, high blood pressure, diabetes mellitus, smoking, overweight and obesity, physical inactivity and family history of premature heart disease (Pohle et al, 2001; Bonow et al, 2006). For example, in the Framingham Study, the odds ratio for aortic valve calcium associated with every standard deviation (SD) increment in long-term mean total cholesterol was 1.74; with every SD increment in HDL cholesterol, it was 0.77; and with every nine cigarettes smoked per day, it was 1.23 (Thanassoulis et al, 2010). Associations of similar magnitude were seen for mitral valve calcium. C-reactive protein has not been associated with either the presence or progression of calcific aortic valve disease (Novaro et al, 2007). Rheumatic fever has declined sharply as a cause of valvular disease in industrialised nations but is still a major burden in the developing world.

2.2.2 Congestive heart failure

Congestive heart failure (CHF) refers to the impairment of the pumping function of the left ventricle of the heart. CHF may result from several underlying conditions including myocardial infarction, cardiomyopathies and valvular heart disease. Several risk factors have been consistently associated with the development of CHF, including age, male sex, left ventricular hypertrophy, diabetes mellitus and hypertension (Bahrami et al, 2008). High body mass index (BMI), even in the pre-obese range, has been associated with an increased risk of CHF, and vigorous physical activity has been associated with a decreased risk (Kenchaiah et al, 2008). The associations of dyslipidemia and cigarette smoking with CHF have been less consistent (Bahrami et al, 2008). Recently, elevated inflammatory markers have been shown to independently predict incident heart failure in several cohorts (Kalogeropoulos et al, 2010). Population attributable risk, determined from predictors of risk and prevalence in an elderly cohort, was relatively high for prevalent coronary heart disease (13.1%), systolic blood pressure higher than 140 mm Hg (12.8%) and a high level of C-reactive protein (9.7%), but was low for subnormal left ventricular function (4.1%) and atrial fibrillation (2.2%) (Gottdiener et al, 2000).

2.2.3 Cardiac arrhythmias

Atrial fibrillation (AF) is the most common cardiac rhythm disturbance. Arrhythmias are more common in people who have diseases or conditions that weaken the heart, such as coronary heart disease, cardiomyopathy, heart failure, valvular disease and congenital heart defects. In the Framingham Study, heart failure imposed the greatest risk of AF, with a 4.5-fold increased risk in men and a 5.9-fold increased risk in women (Kannel et al, 1998). The incidence of AF is approximately double for each advancing decade of life and, at any given age, men have a 50% higher incidence of AF than women (Benjamin et al, 2009). Other conditions that have been shown to increase an individual's risk of developing cardiac arrhythmias include diabetes and high blood pressure; diabetes and high blood pressure increased the risk of developing AF by approximately 40% and 50%, respectively, in participants of the Framingham Study. Also, infections that damage the heart muscle or the pericardium, obstructive sleep apnoea and thyroid disease (both hyperthyroidism and hypothyroidism) increase the risk of developing AF and other cardiac arrhythmias (Gami, 2007; Klein and Danzi, 2007).

2.2.4 Cardiomyopathies

Cardiomyopathies are a heterogeneous group of diseases of the myocardium. They are divided into two major groups based on predominant organ involvement: primary cardiomyopathies (genetic, non-genetic and acquired), which are solely or predominantly confined to heart muscle, and secondary cardiomyopathies, which show pathological myocardial involvement as part of generalised systemic disorders (Maron et al, 2006). The major types include dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy. A familial cause has been shown in 50% of patients with hypertrophic cardiomyopathy, 35% with dilated cardiomyopathy, and 30% with arrhythmogenic right ventricular cardiomyopathy (Franz et al, 2001). Cardiomyopathy risk factors include heart failure or coronary heart disease, family history of cardiomyopathy, myocarditis, diseases that can damage the heart such as haemochromatosis and amyloidosis, prolonged excessive intake of alcohol, prolonged high blood pressure and diabetes. Certain drugs (such as cocaine and amphetamines) and medicines used to treat cancer (eg doxorubicin and daunorubicin) have also been linked to dilated cardiomyopathy (National Heart, Lung and Blood Institute, NHLBI, 2010).

2.2.5 Myocarditis and pericarditis

Myocarditis and pericarditis are inflammation of myocardium and pericardium, respectively. Myocarditis and pericarditis can result from viral, bacterial or fungal infections, myocardial infarction, cancer spreading from a nearby tumour in the lung, breast or the blood, and injury or surgery (AHA, 2010b).

2.3 Summary

1 The underlying cause of common cardiovascular diseases such as coronary heart disease and stroke is, in the majority of cases, atherosclerosis.

2 Non-modifiable risk factors for cardiovascular diseases include age, sex and genetic predisposition.

3 Traditional well-established modifiable risk factors include high blood pressure, presence of diabetes, high total and LDL blood cholesterol, low HDL cholesterol and smoking.

4 Lifestyle factors such as diet, physical inactivity and overweight have been associated with cardiovascular risk factors (diabetes, blood pressure and blood cholesterol levels) and cardiovascular disease.

5 Emerging risk factors including inflammatory markers have shown consistent associations with cardiovascular diseases, but there are as yet no clinical trial data on treatment of these biomarkers and reduction of cardiovascular disease.

6 Other circulatory diseases do not have as strong contributory risk factors as atherosclerotic cardiovascular disease.

3 Radiation Exposure of the Circulatory System

3.1 Public and Occupational Exposures

The average annual effective dose to a member of the public in the UK is 2.7 mSv (Watson et al, 2005), the majority of which is attributable to natural background radiation. There are many sources of public exposure, some of which are internal and some external; some types of radiation are highly penetrating, generally low linear energy transfer (LET) radiations, while others are weakly penetrating, generally high LET radiations or low LET beta particles from radionuclide decays. External penetrating sources will result in exposure of the vasculature including that of the brain and heart; internal sources such as potassium-40 will expose the vasculature. The major contributor to the average effective dose to the UK population, radon (accounting for about 50% of the average effective dose), is unlikely to deliver significant doses to the brain or heart vasculature but will deliver doses to the lung vasculature. Therefore the average UK citizen probably receives annual absorbed doses of the order of 1 mGy or less to the vasculature. Based on calculations, absorbed doses to the brain and heart from natural radionuclides ingested into the body are estimated to be about 180 μGy from low LET sources, predominantly potassium-40, and 0.7 μGy from high LET sources, mainly lead-210 and polonium-210 (T Fell, HPA, 2010, personal communication). External photon doses to the brain and heart are estimated to be about 350 μGy (J Jansen and R Tanner, HPA, 2010, personal communication).

Radiation doses to the circulatory system received occupationally will again be influenced by the nature of the radiation being used. External low LET exposures will deliver doses to the vasculature including that of the brain and heart, while external high LET exposures will deliver only very small, if any, doses to the vasculature. An exception to this is the external exposure to neutrons, which are mainly high LET and penetrating. Few workers approach the occupational dose limits, with average occupational effective doses being 0.4 mSv and only 1% of recorded doses exceeding 5 mSv and none exceeding 10 mSv (Watson et al, 2005). So again, occupational doses to the circulatory system will generally be low.

Doses to the vasculature, heart and brain in particular, can be received in the course of medical examinations and treatments that utilise ionising radiation. There are many such medical uses but it will be radiotherapy treatments that deliver the most substantial doses to tissues of concern. Table 3.1 provides the typical absorbed and effective doses from medical examinations; procedures that directly expose the heart or brain will of course deliver a greater dose to the organs of importance for circulatory disease. The doses presented in Table 3.1 are typical of those delivered in current UK practice. They are based on the average values of adult patient doses in the 2005 review of the UK National Patient Dose Database (Hart et al, 2007) or the 2003 survey of CT doses in the UK (Shrimpton et al, 2005).

Some well-characterised specific incidents have led to relevant radiation exposures in this context. The atomic bombings in Japan led to a mean effective dose of 200 mSv (Brenner et al, 2003). Accidental exposures could of course lead to significant doses also; the Chernobyl nuclear plant incident led to mean cumulative whole body doses of 100 mSv for emergency workers (liquidators), between 10 and 50 mSv for evacuees and residents of strict control zones, and 7 mSv for other people living in contaminated areas (World Health Organization, WHO, 2006); these again are quoted in terms of effective dose.

TABLE 3.1 Typical heart, brain and effective doses for six common X-ray examinations in the UK
Effective dose based on ICRP Publication 103 (ICRP, 2007) methods for calculation (A-P – anterior-posterior, PA – posterior-anterior, Lat – lateral)

Examination	Brain dose (mGy)	Heart dose (mGy)	Effective dose (mSv)
Head (AP+PA+Lat radiographs)	0.68	0.0008	0.068
Chest (PA radiograph)	0.0002	0.016	0.014
Barium swallow (radiography/fluoroscopy)	0.028	3.4	1.5
Coronary angiography (radiography/fluoroscopy)	0.0072	13	3.9
Head CT scan	45	0.020	1.4
Chest CT scan	0.14	13	6.6

3.2 Radiation Dose to the Cardiovascular System with Special Reference to the Heart during Radiotherapy

The role of very high dose therapeutic radiation in causing late vascular insufficiency and damage is well established. Doses over 45–50 Gy when fractionated at 1.8 to 2 Gy per day can cause such effects, as can lower doses given in larger fractions. Clinicians with responsibility for prescribing radiation (clinical oncologists in the UK) were in the past cautious about irradiating limb peripheries at very high dose, such as between 60 and 70 Gy, because of the risk of end-arterial occlusion (eg Arnott, 1986). Although the pathological appearances of irradiated tissues usually show characteristic vascular changes at these very high doses – wherever radiation is given in the body – there is little in the way of guidelines that would limit radiation doses to connective tissues that are below those used to eradicate cancers (ie fractionated doses of 45–50 Gy and more), apart from specific organ-related limits such as those to the lens of the eye, kidney and lung. Gradually with time unnecessary irradiation of the heart has been questioned because of the risk of causing cardiac damage (Taylor et al, 2007).

3.2.1 Cardiac irradiation during radiotherapy

Radiation exposure of the heart occurs during the treatment of tumours in the thoracic region, such as lung cancer, oesophageal cancer, mediastinal lymphoma and thymoma, primary and secondary spinal, para-spinal or bone tumours, and breast cancer. Of these, the most commonly occurring are cancers of the lung and breast. Up to 40% of the population are at risk of developing cancer and around the same percentage go on to receive radiotherapy at some stage in their illness. Irradiation of the thorax is not therefore a rare occurrence and it has been shown that radiotherapy can cause a wide variety of cardiac dysfunction, including acute and chronic dysrythmias, coronary artery disease and cardiomyopathy, all of which may be life-shortening (Yahalom and Portlock, 2008). Corrective medical or surgical therapy – where feasible – will also pose serious risks.

The effect of radiation on the heart is made more complicated to analyse in oncology because many forms of cytotoxic chemotherapy can be toxic to the heart. Drugs of the anthracycline and vinca alkaloid classes are especially cardiotoxic and the cumulative risk continues to be refined for different types of drug (Swerdlow et al, 2007; Ryberg et al, 2008). The combined effect of radiotherapy and drug therapy can potentiate each other even when not administered simultaneously, but in the case of biological agents such as monoclonal antibodies used for cancer therapy, there is no evidence as yet of enhanced radiation cardiac toxicity over ten years, despite these drugs causing a low incidence of cardiac side-effects in 10–20% of patients. For example, the use of Herceptin does not appear to cause enhanced acute cardiac toxicity when used alongside left-sided breast radiotherapy (Shaffer et al, 2009). However, there are insufficient long-term follow-up data available to make a definitive judgement on the potential interactions between biological medicines such as Herceptin and radiation in causing cardiac toxicity.

3.2.1.1 Choice of treatment

The medical decision to treat cancer using radiation is made on the basis that the benefits in terms of tumour control or symptom relief should outweigh the disadvantages of radiation-induced side-effects, and that no other treatment modality can offer a better alternative. This is judged by each clinical oncologist on the basis of the expected prognosis and behaviour of the particular tumour, along with the selection of dose and its fractionation. In some instances, because of adverse dose distributions, it may be necessary to modify the local protocol by reducing the prescribed dose and/or by using a more forgiving fractionation schedule.

Radiotherapy is given with either curative (radical) or palliative intent. Curative treatment usually indicates the use of very high doses with expectation of prolonged survival to beyond five years in a reasonable proportion of patients; for palliative treatment lower doses are given for relief of symptoms in incurable situations associated with a very low probability of prolonged survival. In general, much simpler techniques are used for palliative treatments: for example, the centrally situated carcinoma of the bronchus may be irradiated by an anterior and posterior (opposed) megavoltage X-ray field arrangement, which delivers a broadly uniform dose to all mediastinal structures, with islands of higher dose at around 2–4 cm depth. But even in the case of mediastinal lymphoma, those treated radically by radiotherapy

have usually received significant doses of cytotoxic chemotherapy and opposed radiation fields encompassing most of the heart.

Typical examples of prescribed radical and palliative treatment absorbed doses for two classes of malignant tumours are shown in Table 3.2. The lymphomas are of higher intrinsic radiosensitivity than epithelial cancers, and can be cured with lower doses. In general, radiation prescriptions for this condition were routinely given on the assumption that the heart would tolerate doses of 40 Gy of well-fractionated radiation.

TABLE 3.2 Examples of prescribed radiotherapy treatment doses
The numbers refer to absorbed dose in gray with fraction numbers enclosed in parentheses

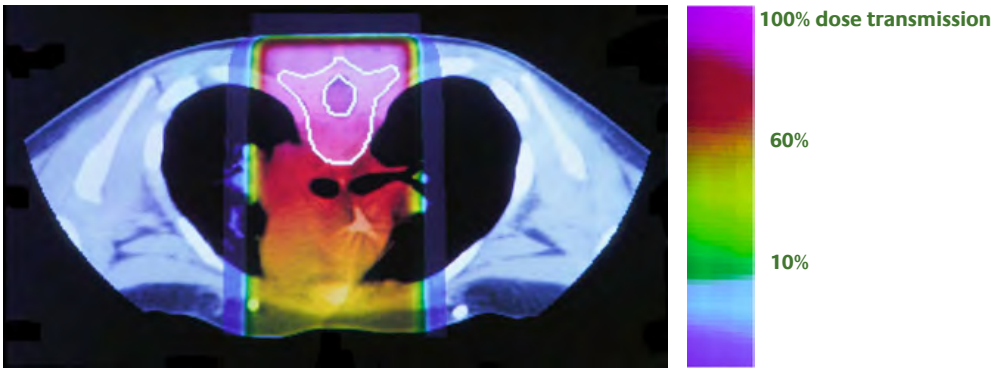
Non-small cell lung cancers		Lymphomas	
Radical	**Palliative**	**Radical**	**Palliative**
60 (30)	30 (10)	30–40 (15–22)	20–30 (10–15)
50–55 (20)	20 (5)	–	15 (5)
40–45 (15)	16–18 (2)	–	2–6 (1)

For radical treatments, a more sophisticated technique is necessary to deliver a higher dose with some degree of sparing of surrounding tissues. For example, a three- or four-field technique can be used to deliver a higher dose where the beams overlap in the locality of a cancer, but even then the heart and lung may be exposed to the non-intersecting portions of the beams which inevitably pass through the entire thickness of the thorax and may cause partial cardiac irradiation. To further improve dose conformity and to reduce dose to critical organs such as spinal cord, the use of intensity modulated radiotherapy (IMRT) is presently increasing in the UK. This technique uses non-uniform radiation fluence over multiple beams, which can increase to six to nine beams in some instances. This essentially transfers energy deposition from a critical area to another of lesser importance. For instance, in this way spinal cord dose can be reduced during thoracic irradiation, but at the expense of dose transfer to other tissues which could be the lung or the heart. Dose constraints can be applied to each normal tissue and compromise solutions achieved. In some studies cardiac dose can be substantially reduced – for example, the volume of heart receiving more than 30 Gy from 12.5% to 1.7% – but at the expense of dose to other normal tissues including the contra-lateral breast (Beckham et al, 2007). In general, tissue dose sparing becomes more difficult to achieve as the number of tissue constraints used in the treatment planning process increases.

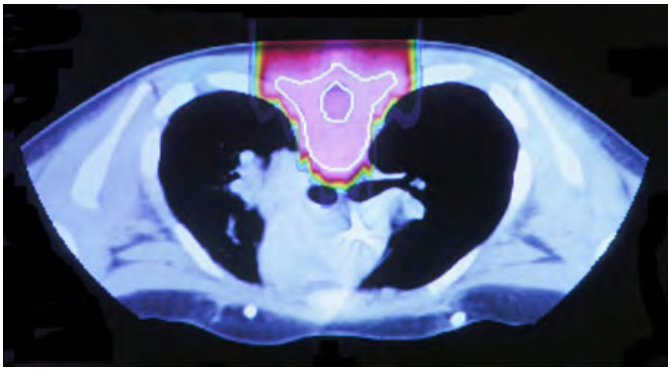
3.2.1.2 Elective irradiation of the heart

It is necessary to include cardiac tissues in the very high to moderate treatment dose region in some clinical situations. For example, for a large mediastinal lymphoma/thymoma or carcinoma of the bronchus, the cancer can invade the pericardium and also infiltrate cardiac structures such as the conducting tissues and muscle, so that adequate cover of the heart to what is judged to be a reasonable dose for sterilisation of microscopic tumour cell numbers is indicated. This may amount to at least 30 Gy for lymphomas and higher doses such as 50 Gy or more for carcinomas.

The use of intracoronary beta-radiation therapy to inhibit occlusive coronary artery disease was led by enthusiastic cardiologists, but received caveats from radiobiologists concerned with vascular damage and aneurysm formation (Jones, 1999). So far, the clinical results have not been impressive despite considerable activity in some countries (Vlachojannis et al, 2010).



A Megavoltage X-rays



B Protons

FIGURE 3.1 Medulloblastoma spinal treatment plans using either (A) X-rays or (B) protons, as used at the Massachusetts General Hospital. The colour wash dose-scale shows the extent of dose transmission through the body for X-rays compared to none for protons. Courtesy of Dr J Munzenrider, Massachusetts General Hospital; for further details see St Clair et al (2004)

3.2.1.3 Spinal tumours

The treatment of spinal tumours is worthy of special mention as the dorsal spine is normally regarded as distant from the heart. A divergent megavoltage X-ray beam aimed from a posterior direction over the lower five vertebrae will exit through most of the heart, sparing only the lateral aspect of the left ventricle. One such example is shown in Figure 3.1 for medulloblastoma of children and young adults. Such cardiac irradiation may not be of great significance for a patient with metastatic cancer and a life expectancy of, say, 6–12 months, but in the case of a patient with a spinal tumour associated with a good prognosis, cardiac dose may be significant.

3.2.1.4 Gating techniques

The use of respiratory gated radiotherapy, where treatment fields and in some instances exposure pulses are dynamically adjusted to match respiratory movements, is receiving greater attention (Ding et al, 2007). This technique is in routine use for treatment of some cancers (hepatoma, lung cancer and oesophageal cancer), especially using charged particles (see below), but they can also be used with X-ray techniques such as IMRT. In studies of pure respiratory gating for breast cancer, the cardiac doses did not change significantly (Ding et al, 2007) as might be expected, since specific cardiac gating would be required. Such a technique could, in principle, be used to reduce cardiac dose only if pulsed radiation is synchronised to the cardiac contraction cycle.

3.3 Special Case of Breast Cancer

The future medical management of left-sided breast cancers poses a special dilemma, since chest wall radiotherapy – encompassing the whole breast – is presently given to around 150 such patients per million of population each year. It has been shown that standard megavoltage X-ray beams can directly traverse a small portion of the anterior left ventricular wall and consequently encompass the left anterior descending coronary artery.

The standard beam positioning for a breast cancer includes a medial tangential field placed just beyond the midline and the lateral (opposing) field at the mid-axillary line (see Figure 3.2). It is sometimes possible to modify these positions depending on coverage of the breast tissue, the tumour position and need to cover the internal mammary lymph node chain of lymph nodes in order to reduce lung and heart traversal, but in most instances only relatively trivial adjustments are possible. In some cases, such as cancers situated in the medial part of the breast, additional radiation can be given by means of a further direct field, using the rapid fall-off characteristics of electron beams to spare underlying cardiac tissue. The anatomical shape of the chest wall and manubrium will also affect the local treatment geometry, so that individuals with congenital or acquired chest wall deformities, such as *pectus excavatum*, may have a greater proportion of the heart irradiated. Patients with cardiomegaly, for whatever cause, may also have excessive volumes of heart irradiated if only standard techniques are used.

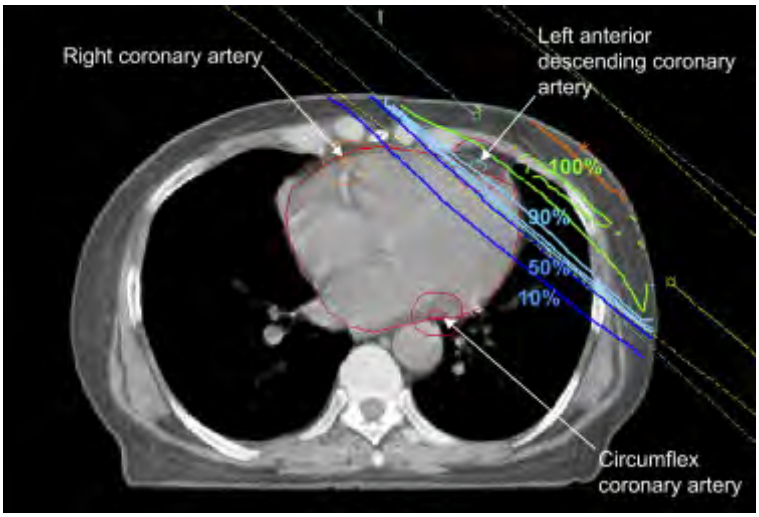


FIGURE 3.2 Examples of potentially significant cardiac irradiation using two megavoltage (cobalt-60) tangential fields across the chest wall, as used in post-mastectomy breast cancer patients. The position of the visible coronary arteries is shown and the left anterior descending coronary artery is directly irradiated, with lower doses to the deeper adjacent myocardium. (From Taylor et al, 2007)

The dose prescription for radical breast treatments in the UK has varied between 50 Gy in 25 fractions to 40 Gy in 10 fractions over many years, but with 40 Gy in 15 fractions replacing the latter. These doses are not homogeneous and it is not unusual to obtain large variations above the prescribed dose (Neal et al, 1994) unless special compensatory techniques (Evans et al, 1995) or IMRT (Beckham et al, 2007; Ding et al, 2007) are used. It has been estimated that partial cardiac irradiation to over 20 Gy occurs during irradiation in over half of left-sided breast cancer patients (Taylor et al, 2009a) when standard techniques are used.

3.4 Alternative Techniques to Standard External Megavoltage X-rays

Cardiac radiation exposure can be minimised or completely avoided by use of alternative forms of radiation or new radiotherapy technology such as helical tomography. These are discussed below.

3.4.1 Brachytherapy and intraoperative X-rays

Brachytherapy essentially means short range therapy by temporary insertion of sealed radionuclide sources in the tumour-bearing region. The radiation fall off with distance (d) is much greater than with external radiation beams, because of the inverse square law effect ($1/d^2$) from each point source of radiation used. This becomes a relationship of $1/d$ for a very long linear arrangement of sources, so that in most practical examples the fall off is intermediate and can be approximated by an exponential function, eg dose = $e^{-0.7d}$. The approximate percentage doses at 1, 2, 3, 4 and 5 cm from the centre of an implant would then be around 50%, 25%, 2%, 6% and 3%, respectively. Cancers of the bronchus,

oesophagus and breast may be treated in this way for palliation or, in some instances, radical purposes. For breast cancers the 20 Gy volume of cardiac tissues is significantly reduced by a local brachytherapy technique compared with external radiotherapy alone (Stewart et al, 2008), but it remains unclear whether it is satisfactory to use brachytherapy only in the treatment of breast cancer because of the risk of local relapse in another part of the breast. Other forms of localised radiotherapy to only a small volume of breast tissue are available, such as intraoperative kilovoltage X-rays (Vaidya et al, 2010) but the patient follow-up duration of only four years is relatively short so that the conclusions presented may require revision at a later time.

3.4.2 Charged particle therapy (CPT)

In contrast to the highly penetrating high energy X-rays used to treat most cancers, charged nuclear particles decelerate through tissue and form a dose peak at a depth controlled by their energy and atomic mass, with little or no radiation beyond the peak. CPT allows the oncologist to treat a cancer more selectively with much reduced energy deposition to surrounding tissues (Jones and Akine, 2006; Schultz-Ertner and Tsujii, 2007; Jones, 2008; Zhang et al, 2009). The dose distribution advantage in a child with medulloblastoma is shown in Figure 3.1 and for irradiation of the whole breast for cancer in Figure 3.3. A comparison of dose profiles to the heart in oesophageal cancer treatment using protons or X-rays is given in Table 3.3 and the advantageous dose distributions for proton therapy can be seen in Jones (2008). The cost of CPT is presently higher than that of X-rays by a factor of three and so facilities are limited worldwide, although there is a major expansion of facilities in many countries. The UK government has recently announced plans for NHS particle therapy facilities. These proton beam centres will provide scope for reducing cardiovascular irradiation in many clinical situations.

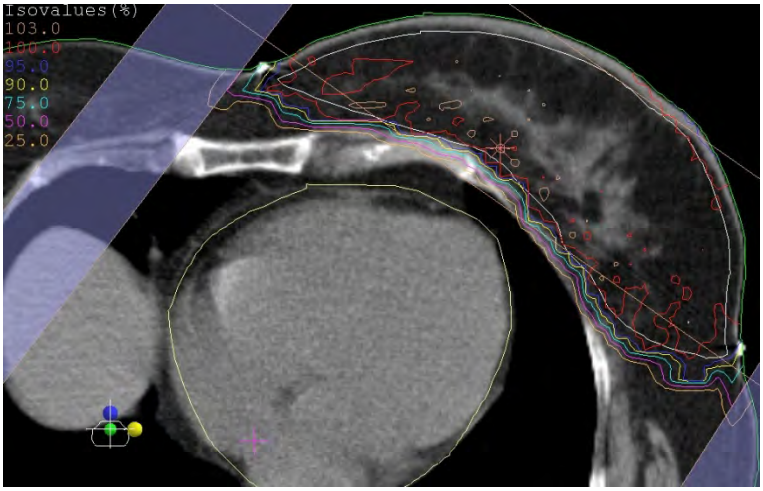


FIGURE 3.3 Left breast of a breast cancer patient treated entirely by protons delivered from a single field. The particle beam does not irradiate any part of the myocardium and the left anterior descending coronary artery is spared in this case, in contrast to the situation shown in Figure 3.2 where X-rays were used. Dose distribution is indicated by coloured isodose contours – see top left of figure for scale. (From Jones et al, 2009)

TABLE 3.3 Comparative cardiac absorbed doses for X-rays and protons (data taken from Jones and Akine, 2006). Better distributions will be found for beams which do not traverse the heart, but at the expense of increasing the lung dose. The same principles apply to lung cancer therapy

	4-field X-rays (UK)	2-field protons (Japan)
Prescribed dose	54 Gy (30 fractions) + chemotherapy	72 Gy (36 fractions) – radiotherapy only
Mean cardiac dose	41.4 Gy	18.7 Gy
Heart volume receiving ≥ 30 Gy	77%	15%
Heart volume receiving ≥ 40 Gy	51%	10%
Heart volume receiving ≥ 45 Gy	26%	8%

3.4.3 Helical tomography

Helical tomotherapy uses external megavoltage X-rays and has been developed for use in breast cancer treatment allowing irradiation of the internal mammary chains while minimising heart irradiation (Caudrelier et al, 2009). However, caution may be needed to ensure radiation exposure of other tissues is not unduly elevated.

3.5 Irradiation of the Brain, Head and Neck

Radiation is widely used in the management of malignant tumours and sometimes relatively benign disorders occurring in the brain, head and neck regions. In the last, which includes vascular malformations and some very slow growing tumours, prolonged survival is possible over a 10–30 year duration, which allows manifestation of late radiation effects mediated by vascular damage. The brain, because of its high metabolic activity, is the tissue which experiences the highest blood flow rates in the body: it is consequently at risk of ischaemic changes when its arterial blood supply is compromised. The brain has a complex arterial supply mediated by two arterial systems in the neck: the carotid arteries (the main supply to the cerebral cortex) and the smaller vertebral arteries which pass through the vertebral bones prior to supplying the lower involuntary and vital brainstem regions as far as the occipital cortex, which provides visual appreciation. The carotid and vertebral arteries also merge to form the Circle of Willis, a conjoined ring of arteries which shares these two input systems. Thus severe injury, such as occlusion of, say, one carotid artery, may not necessarily result in catastrophe such as a severe stroke unless there is also severely compromised flow in the contra-lateral carotid. However, a damaged but partially occluded artery is prone to embolic episodes so might cause stroke. At higher levels in the brain there are critical other regions such as the internal capsule where a bleed may cause severe stroke. Also, the lenticulo-striate artery, which supplies thalamic regions that control some aspects of motion, is an end-artery without anastomotic connections. Anastomoses refer to an overlapping system of blood supply to a tissue from different arteries such that damage to one supply can at least partially be overcome by an expansion in flow from the other blood vessels.

Other vascular systems in the brain are very susceptible to radiation damage, such as the hypothalamic-pituitary portal system which transports releasing factor hormones between these two important locations. This system is susceptible to damage from doses down to 20 Gy of fractionated radiotherapy, resulting in pituitary gland insufficiency (Darzy and Shalet, 2009). Vascular damage in the brain can be devastating – even the development of blood vessel telangiectasiae, which cause innocent bleeding in some areas of the body, can initiate intra-cerebral bleeding.

Irradiation of the neck can result in carotid arterial occlusion, especially in persons with already compromised carotid blood flow due to acquired carotid stenosis. There is also an enhanced risk of embolic-strokes from irradiated atheromatous carotid artery regions. There is evidence of such an effect following radiotherapy of head and neck cancers and lymphoma (Dorresteijn et al, 2002; Haynes et al, 2002; Rogers, 2003), although Rogers (2003) also considers the issue of hypercoagulation in cancer patients. Even in the case of relatively low stage (small volume) laryngeal cancers, carotid irradiation can be avoided by using lateral beams rather than anterior oblique fields which transmit dose deeper into the neck. For larger and deeper tumours carotid irradiation may be unavoidable; indeed the cancer may be surgically inoperable on the basis of its proximity or adherence to an artery such as the carotid.

A further issue is the use of intensity modulated radiotherapy which can achieve a good reduction in dose to critical organs such as the spinal cord, but displace dose due to 'fluence-transfer' to connective tissues, which may include major arteries. Ideally the treating physician should ensure that constraints should be applied to areas of the vascular tree which are not thought to be involved in the cancer. In this respect, reduction of the treatment volumes to what is absolutely necessary appears to be a worthwhile aim and can be achieved to an extent with more detailed three-dimensional treatment planning techniques.

A major issue is that an increasing number of patients receive neo-adjuvant or concomitant chemotherapy with radiotherapy for squamous cell carcinomas, due to the medical evidence that tumour control and disease-free survival are improved (Adelstein et al, 2003; Cooper et al, 2004). However, the most severe normal tissue complications following high doses of radiation and chemotherapy appear to be worse, with examples of carotid artery rupture and sudden death.

3.6 Abscopal Irradiation Effects

Adverse effects have been described on the vascular system due to remote (abscopal) mechanisms following irradiation of non-vascular tissues. Examples include brain irradiation causing pituitary insufficiency resulting in hypothyroidism with its own cardiovascular effects (low pulse rate, hypercholesterolaemia and atheromatous complications). Similarly, irradiation of the kidney(s) can cause severe hypertension resulting in cardiac failure (this is mediated by increased angiotensin II, which can be reduced by angiotensin converting enzyme (ACE) inhibitor class drugs. Elevated serum TGF-β (transforming growth factor beta) has been identified as a predictive marker of radiation-induced fibrosis (Anscher et al, 1997). While there is no direct evidence, it may be that the high serum TGF-β signal plays some role in acceleration of lung and cardiac fibrosis. It is self-evident that radiation lung damage (fibrosis)

which results in reduced gas exchange and tissue hypoxia will cause additional cardiac demand, which may precipitate heart failure and reduce the threshold to the development of dysrhythmias. Also, the additional resistance offered to blood flow by severe lung fibrosis due to radiation could be sufficient to cause right-sided heart failure, known as *cor pulmonale*. Chapter 6 provides a fuller account of cytokine signalling of fibrosis and inflammation following radiation exposure.

3.7 Diagnostic Procedures during Radiotherapy and Elsewhere

There is increasing use of additional diagnostic ionising radiation to improve the accuracy of radiotherapy: this is often referred to as image-guided radiotherapy. It is advised in some instances that regular CT scans should be performed sometimes on a daily basis, especially during the early phase of treatment, in order to determine precise organ positions so that the treatment field sizes and positions can be adjusted to match the scan appearances: this is referred to as 'adaptive' radiotherapy. Linear accelerators used to deliver cancer treatment can now be fitted with 'cone beam' CT systems that allow imaging immediately prior to therapy. There has been a debate about permissible dose from such imaging procedures which can sometimes deliver a total body dose (Green and Aird, 2007; Harrison et al, 2007; Harrison, 2008), although most physicians would justify such use on the basis of improved radiation delivery to a cancer. Increasing doses from diagnostic radiology in general (rather than specifically linked to radiotherapy) have been noted in recent years and the health impact of this change in practice has yet be fully appreciated (eg Brenner and Hall, 2007; Bhatti et al, 2008; Gerber et al, 2009; Hausleiter et al, 2009). In particular, concerns have been raised about the use of CT scanning of asymptomatic individuals (COMARE, 2007). Any exposure of the heart or brain during such diagnostic procedures may be contributing to circulatory disease risk as well as cancer risk.

3.8 Summary

1 Public and occupational doses to the heart and brain are generally low, medical diagnostic procedures in general also deliver low doses, head and chest CT examinations can lead to absorbed doses of several tens of milligray each.

2 Radiotherapy probably accounts for more very high dose vascular radiation exposure than occurs during occupational or other forms of exposures, including cardiac diagnostic procedures.

3 A variety of methods are available to allow cardiac and other vascular exposures to be reduced but it remains important for clinicians to minimise normal tissue exposures wherever possible while maintaining clinical therapeutic benefit.

4 Epidemiological Studies of Circulatory Disease in Irradiated Human Populations

4.1 Technical Issues in the Evaluation of Epidemiological Studies

The availability of mortality data, recording age, sex, area of residence and cause of death, is almost complete for the UK for deaths since 1959. These data are collected by the General Register Offices for England and Wales, Scotland and Northern Ireland. However, there are known to be generally quite marked errors in attributing cause of death on death certificates. For stroke, the accuracy of diagnosis is reasonably good. Out of a sample of 250 cases of stroke identified between 1993/97 and 2003 using ONS record linkage in the EPIC-Norfolk study, review of hospital notes revealed that there were 191 definite strokes (76%), 20 probable strokes (8%), 11 possible strokes and 11 cases of transient ischaemic attacks (4% each) – 233 out of 250 cases (93%) with possible or definite stroke or transient ischaemic attacks (Sinha et al, 2008). The diagnosis of stroke in 185 out of 250 cases identified in the EPIC-Norfolk (74.0%) was supported by radiological evidence using WHO criteria (Sinha et al, 2008). That said, there may be substantial misclassification for other types of cardiovascular disease. For example, only 54% agreement was found between blinded panels of clinicians (comprising pairs of pathologists and cardiologists) of clinical information from autopsy reports on 400 cardiac deaths randomly selected from a defined population in the West Midlands (Mant et al, 2006). It is known that some cardiovascular deaths are coded as asthma: an expert review of 2382 deaths found that 2 of 22 deaths coded asthma were really cardiovascular disease (Guite and Burney, 1996).

The same is true in many other developed countries. The validity of death certificate diagnosis of stroke and its type as the underlying cause of death was investigated in a sample of in-hospital deaths of possible stroke cases in Minnesota, USA (Iso et al, 1990). The 228 in-hospital deaths in 1970 and the 180 deaths in 1980 had a stroke diagnosis either on hospital discharge records or as the underlying cause of death on the death certificate. Relative to a standardised physician diagnosis, positive predictive values for the death certificate diagnosis in 1970 were 96% for all types of stroke, 59% for intracranial haemorrhage and 87% for non-haemorrhagic stroke. The respective values in 1980 were 100%, 82% and 97% (Iso et al, 1990).

The Radiation Effects Research Foundation (RERF) has some (quite old) data on the accuracy of cardiovascular disease death certificate data in comparison with autopsy data for the Life Span Study (LSS) of the survivors of the atomic bombings in Japan (the LSS cohort). These were collected in the period up to the early 1970s. Presumably, the accuracy of circulatory disease death certificate causes would be greater today than it was for this period, because of improved diagnostic methods and technologies. For 688 death certificates listing stroke as the primary cause of death in the comparison, 86% of autopsies listed stroke as one of the conditions found (Shimizu et al, 2010). However, only 40% of the 688 stroke

death certificates listed stroke as the primary cause of death on autopsy review (Ron et al, 1994). Ron et al indicate that for 62% of those not listing stroke as the primary cause at autopsy, the autopsy primary cause was heart or other circulatory disease. For 33% of those listing stroke as the primary cause of death on the autopsy report, stroke was not the primary cause on the death certificate. For 1236 death certificates listing heart disease as the primary cause of death in the comparison, 92% of autopsies listed heart disease as one of the conditions found (Shimizu et al, 2010). However, only 52% of the 1236 heart disease death certificates listed heart disease as the primary cause of death on autopsy review (Ron et al, 1994). For 66% of those listing heart disease as the primary cause of death on the autopsy report, heart disease was not the primary cause on the death certificate. The "accuracy [ie percentage of death certificate diagnoses where that disease was listed on the autopsy report] was rather poor for the differential diagnosis of specific subcategories of stroke or heart disease (for example, 65% for cerebral infarction, 39% for cerebral hemorrhage, 69% for ischaemic heart disease, 22% for hypertensive heart disease, and 64% for rheumatic heart disease)" (Shimizu et al, 2010).

The Japanese family registration, or *koseki* system, deserves special mention. In brief, under Japanese law, each mayor or village head is required to maintain a *koseki* for each family whose *honseki* (a kind of legal residence) is located within his jurisdiction. Every death or birth to a person whose name is inscribed on the *koseki* must be posted to this registry, with the name deleted or a new name added, as required. Similarly, marriages result in the deletion of the names of the newly married pair from their parents' *koseki* and establishment of a new *koseki* for the newly created family. Japanese law requires that copies of certificates of birth or death, or notifications of marriages be sent to the appropriate *koseki* offices as a basis for posting changes. Completeness of birth registration, in particular, is virtually guaranteed by the requirement of *koseki* registration for a child to be admitted to school. Similarly, the fact that a cremation or burial permit is issued only after the death has been registered ensures a high level of completeness in the posting of deaths to the *koseki*.

Tests have been made of the completeness of mortality recording under this system, which imply that completeness of death registration is about 99.3% (Beebe et al, 1962). As in the UK, the accuracy of diagnosis of various types of cardiovascular disease is variable, better for stroke than for other endpoints. In a study of specific diagnosis for cardiovascular diseases in death certificates made at autopsy in 864 consecutive autopsy cases aged 20 years or over among residents in Hisayama, stroke was correctly diagnosed in 84% and cardiac disease in 66% (Hasuo et al, 1989). Cerebral stroke and cardiac disease tended to be overdiagnosed. The validation of certified diagnosis was less reliable in the aged population, and in type-specific diagnosis of cardiovascular diseases. Cerebral haemorrhage with false-negative or false-positive diagnoses was usually classified into type-unspecified stroke or different categories of cerebral stroke, while those misdiagnosed as cases of cerebral infarction frequently had no significant lesions in the autopsied brain (Hasuo et al, 1989).

In the appendix we consider in detail various types of study designs, types of bias and confounding factors; Box 2 provides a summary description of these. As stated there, epidemiology is the study of the distribution and determinants of disease in human populations (MacMahon and Trichopoulos, 1996). In general, apart from randomised controlled trials (which are largely experimental in design), in most epidemiological studies there is always the possibility that biases or confounding factors of various sorts may give rise to spurious results, as discussed in the appendix. Epidemiological studies are commonly of two types: the cohort study and the case–control study.

BOX 2 Types of Epidemiological Study

Cohort study

Identify a group of persons (a cohort) with individual data on exposures and possible confounders, then follow-up the cohort to determine the relationship between the subsequent disease incidence/mortality and the exposure (eg LSS of the survivors of the atomic bombings in Japan: Shimizu et al, 2010).

Case–control study

Assemble groups of individuals with a given disease (cases) and without disease (controls), then compare the cases and controls with respect to their past exposures to determine the relationship between the disease incidence/mortality and the exposure (eg the RACE study: www.race.ki.se).

Correlation study

A particular type of cohort study but the analyses are based on data averaged over groups of people rather than individuals.

Case–cohort study

Within an existing cohort, information is collected on all cases with a certain disease status as well as on a sample of persons from the underlying cohort. The data are analysed in the same way as a cohort study (eg IRSCCP: Hutchison, 1968).

Randomised controlled trial

People are assigned at random to various groups before planned exposure/treatment and these groups are then followed up to assess their response to the exposure/treatment over some defined period (eg radiotherapy treatment: Bolla et al, 2005).

In a 'cohort study', a defined population (preferably with a wide range of radiation exposures) is followed forward in time to examine the occurrence of many possible health endpoints. Such a study can be performed either prospectively, by following a current cohort into the future, or retrospectively, by using registers to construct a cohort of persons alive at some time in the past, and then following it forward, possibly to the current time and beyond. A 'correlation study' is a particular type of cohort study that is based on data averaged over groups and, in particular, uses data grouped on exposure. However, correlation studies are frequently cross-sectional in nature and, as such, are not strictly cohort studies at all. Typically the imputed exposure for a group may not reflect the disease prevalence in the group, because of in- and out-migration, resulting in a species of ecological bias. For these and other reasons correlation studies are generally regarded as much less reliable than cohort studies. In a 'randomised controlled trial' (RCT), people are assigned at random to various groups before planned exposure to radiation, and these groups are then followed to assess their response to the treatment over some defined period. An RCT may be regarded as a special form of cohort study; however, its essentially experimental design, as opposed to the more observational design of most cohort studies, should be noted.

In a 'case–control study', data on persons with some specified disease (eg some class of cancers) are assembled (the 'cases') together with data on a suitably matched (eg by age and sex) set of persons

otherwise similar to these cases but without the disease (the 'controls'). These two groups are then compared to assess differences in the distribution of a number of exposure variables. The advantage of a case–control study is that detailed histories of radiation exposure and other information (eg history of smoking), which may be difficult to collect for a cohort, can be collected relatively easily for the specific cases and controls. As noted in the appendix, a particular problem with retrospectively assembled case–control studies is recall bias: if exposures are ascertained from the cases or their relatives, recall can be differential dependent on disease status, resulting in bias.

4.1.1 Biases in epidemiological studies

'Bias' in a study may be defined as any process at any stage in the conduct of the study that tends to produce results or conclusions that differ systematically from the truth. One sort of bias is 'follow-up bias', which arises when there is a lack of follow-up information – for example, if persons have, unknown to the investigator, migrated outside the study area, so that their health status cannot be reported. In this instance, they still apparently contribute to the number of person-years (PY) of follow-up in the study, but in reality there is no chance of observing any detrimental effect to their health, making them appear 'effectively immortal'. This form of bias applies equally to cohort studies and case–control studies. Related to follow-up bias is 'ascertainment bias', also sometimes known as 'selection bias', which arises when there is variation in ascertainment of disease status, perhaps correlated with exposure variables. For this reason, much the strongest studies are those that rely upon independently maintained registers of disease and health status, eg the mortality and cancer incidence registers maintained in many developed countries. Ascertainment bias applies equally to both cohort and case–control studies.

It is sometimes necessary to approach cohort members, or their relatives, to recall exposures. This is very likely to be the situation when studies, in particular case–control studies, are organised retrospectively. 'Recall bias' arises when information – for example, on exposure – is collected retrospectively, and cases or their relatives are subject to differential recall of this information, depending on their disease status. For this reason, the strongest studies are those that rely upon independently maintained registers of exposure. Related to recall bias is 'investigation bias', which results if investigators scrutinise exposures more thoroughly for cases than for controls. Although register-based studies are not prone to recall or investigation bias, they are subject to errors due, for example, to inaccurate diagnostic information.

A 'confounding factor' is one that is correlated both with the disease under study and with an exposure of interest. Confounding factors can lead to bias. In many studies there is no reason to expect correlations between most factors and the radiation exposure, so that confounding ought not to be a problem. In studies of medical exposures, confounding may arise if the clinical indications that lead to the exposures are related to a subsequent diagnosis of the relevant disease; this is sometimes referred to as 'confounding by indication'. As discussed in Chapter 2, extensive epidemiological research has identified specific risk factors for circulatory disease, which include male sex, family history of heart disease, cigarette smoking, diabetes, high blood pressure, obesity, increased total and low density lipoprotein (LDL) cholesterol, and decreased high density lipoprotein (HDL) cholesterol plasma levels (Wilson et al, 1998; Burns, 2003; Little et al, 2008). For example, the circulatory disease relative risk (RR) associated with heavy smoking commonly exceeds two (Yusuf et al, 2004), of the same magnitude as the RR associated with

exposure to high doses of radiation (Mulrooney et al, 2009). Therefore confounding by factors related to cigarette smoking can bias studies of circulatory disease; bias is even worse for smoking-related cancers, eg lung cancer, where smoking-related RR can exceed ten (Peto et al, 2000; Pierce et al, 2003). When suitable information on these factors is available, confounding factors can be dealt with at the analysis stage, either by incorporation of such factors into the regression model, or by stratifying the data according to levels of the confounding factor.

4.2 Summary of Evidence from High and Very High Dose Medical Studies (Average Dose Above 5 Gy)

At high or very high radiation doses, such as would be received by patients treated with radiotherapy, there are many human and animal data demonstrating risks to the heart and cardiovascular system, reviewed by Adams et al (2003). After high or very high doses a variety of other (so-called deterministic or tissue reaction) effects are observed, resulting from inactivation of large numbers of cells and associated functional impairment of the affected tissue. Among such effects are direct damage to the structures of the heart – including marked diffuse fibrotic damage, especially of the pericardium and myocardium, pericardial adhesions, microvascular damage and stenosis of the valves – and to the coronary arteries; these sorts of damage occur both in patients receiving radiotherapy and in experimental animals (UNSCEAR, 2006). With the exception of pericarditis, which occurs on timescales of months, most of these endpoints occur ten or more years after irradiation (Adams et al, 2003). Heart and coronary arterial doses associated with radiotherapy treatment can be very large for certain groups treated for malignant disease, but a defect of most studies is the absence of individual radiation dosimetry (eg Darby et al, 2003, 2005a; EBCTCG, 2005; Swerdlow et al, 2007), although studies with high quality individual dosimetry are starting to appear (eg Mulrooney et al, 2009; Tukenova et al, 2010). There is also generally little information on concomitant chemotherapy, and it is known that certain sorts of concomitant chemotherapy (eg vincristine and anthracyclines) are cardiotoxic (Swerdlow et al, 2007). Since concomitant chemotherapy exposure is often correlated with radiotherapy dose there is potential for serious confounding of the dose–response. We note the study of carotid stenosis of Shai et al (2009), which is cited in Table 4.1. However, the endpoint is some way removed from the clinically relevant ones of circulatory disease risk, so no further use is made of the results from this study in our meta-analyses (Tables 4.3–4.7).

4.3 Summary of Strength of Association of Cardiovascular Diseases with Ionising Radiation in Medium and Low Dose Studies (Below 5 Gy)

Epidemiological studies in which the mean heart or brain absorbed doses are generally in the 0–5 Gy dose range are outlined in Table 4.1. The studies considered are more or less those documented in the systematic reviews of Little et al (2008, 2009a, 2010); the latter two of which were updated from Little et al (2008) to consider more recent studies, in particular the BNFL worker study of McGeoghegan et al (2008), the third analysis of the UK National Registry for Radiation Workers (Muirhead et al, 2009) and the Mayak worker study (Azizova and Muirhead, 2009).

TABLE 4.1 Excess relative risks, ERR (per Sv) of cardiovascular disease in published medium and low dose (<5 Gy) epidemiological datasets with estimated average radiation dose to the heart and for which quantitative risk assessment is possible (reproduced in part from Little et al, 2008, 2009a, 2010)

Data	Source	Average heart/brain dose (range) (Sv)	Numbers in cohort (person years follow-up)	Endpoint (mortality unless otherwise indicated)	Numbers of cases/deaths	ERR Sv^{-1} (with 95% CI)
Japanese atomic bomb survivors						
Mortality	Shimizu et al (2010)	0.1 (0–4) [a]	86,611 (3,294,210)	Heart disease (ICD9 393–429 excluding 401, 403, 405)	14,018 [b]	0.18 (0.11, 0.25) [a,b]
				Stroke (ICD9 430–438)	12,139 [b]	0.12 (0.05, 0.19) [a,b]
				Circulatory disease apart from heart disease and stroke (ICD9 390–392, 401, 403, 405, 439–459)	5,846 [b]	0.58 (0.45, 0.72) [a,b]
				All circulatory disease (ICD9 390–459)	25,113 [b]	0.15 (0.10, 0.20) [a,b]
				Heart disease (ICD9 393–429 excluding 401, 403, 405)	8,463 [c]	0.14 (0.06, 0.23) [a,c]
				Stroke (ICD9 430–438)	9,622 [c]	0.09 (0.01, 0.17) [a,c]
				Circulatory disease apart from heart disease and stroke (ICD9 390–392, 401, 403, 405, 439–459)	969 [c]	0.02 (−0.18, 0.29) [a,c]
				All circulatory disease (ICD9 390–459)	19,054 [c]	0.11 (0.05, 0.17) [a,c]
Morbidity	Yamada et al (2004)	0.1 (0–4) [d]	10,339 (na)	Hypertension incidence, 1958–1998 (ICD9 401)	5035	0.05 (−0.01, 0.10) [d]
				Hypertensive heart disease incidence, 1958–1998 (ICD9 402, 404)	1886	−0.01 (−0.09, 0.09) [d]
				Ischaemic heart disease incidence, 1958–1998 (ICD9 410–414)	1546	0.05 (−0.05, 0.16) [d]
				Myocardial infarction incidence, 1964–1998 (ICD9 410)	117	0.12 (−0.16, 0.60) [d]
				Occlusion incidence, 1958–1998 (ICD9 433, 434)	440	0.06 (−0.11, 0.30) [d]
				Aortic aneurysm incidence, 1958–1998 (ICD9 441, 442)	184	0.02 (−0.22, 0.41) [d]
				Stroke incidence, 1958–1998 (ICD9 430, 431, 433, 434, 436)	729	0.07 (−0.08, 0.24) [d]

TABLE 4.1 Continued

Data	Source	Average heart/brain dose (range) (Sv)	Numbers in cohort (person years follow-up)	Endpoint (mortality unless otherwise indicated)	Numbers of cases/deaths	ERR Sv^{-1} (with 95% CI)
Low and medium dose radiotherapy and medical diagnostic studies						
Peptic ulcer study	Carr et al (2005)	1.3 (0.0–7.6)	3719 (92,979)	Coronary heart disease (ICD8 410–414) [e]	910	0.10 (−0.12, 0.33)
				Other heart disease (ICD8 400–404, 420–429) [e]	118	−0.16 (−0.49, 0.17)
Ankylosing spondylitis	Darby et al (1987)	0.14 (0.0–4.80) [f]	14,106 (183,749)	Stroke (ICD7 430–434)	231	−2.43 (−4.29, 0.71) [f]
		2.49 (0.0–17.28) [g]		Other circulatory disease (ICD7 400–429, 435–468)	990	−0.01 (−0.12, 0.13) [g]
TB fluoroscopy	Davis et al (1989)	0.84 [h] (na)	13,385 (331,006)	All circulatory disease (ICD8 390–458)	2191	−0.11 (−0.20, −0.01) [h]
Israeli tinea capitis	Shai et al (2009)	0.093 [i] (0.045–0.495)	145+150 [j] (na)	Carotid stenosis	144	46.88 (19.14, 100.32)
Occupational studies						
Canadian nuclear and other workers	Ashmore et al (1998)	0.063 (0.0–>0.4)	206,620 (na)	Circulatory disease (males) (ICD9 390–459)	1708	2.3 (0.9, 3.7) [k]
				Circulatory disease (females) (ICD9 390–459)	243	12.1 (−0.4, 24.6) [k]
Mayak workers	Azizova and Muirhead (2009), Azizova et al (2010)	0.83 (0–5.92) [i]	12,210 (205,249)	Ischaemic heart disease morbidity (ICD9 410–414)	3751	0.109 (0.049, 0.168) [l]
		0.52 (0–127.82) [j]	12,210 (443,350)	Ischaemic heart disease mortality (ICD9 410–414)	1495	0.275 (0.05, 0.501) [m]
		0.83 (0–5.92) [i]	12,210 (197,344)	Cerebrovascular disease morbidity (ICD9 430–438)	4418	0.464 (0.360, 0.567) [l]
		0.52 (0–127.82) [j]	12,210 (197,344)	Cerebrovascular disease mortality (ICD9 430–438)	753	0.155 (0.075, 0.235) [m]

TABLE 4.1 Continued

Data	Source	Average heart/brain dose (range) (Sv)	Numbers in cohort (person years follow-up)	Endpoint (mortality unless otherwise indicated)	Numbers of cases/deaths	ERR Sv^{-1} (with 95% CI)
Chernobyl emergency workers	Ivanov et al (2006)	0.109 (0–>0.5)	61,017 (na)	Hypertension (ICD10 I10–I15) morbidity	15,484	0.26 (−0.04, 0.56)
				Ischaemic heart disease (ICD10 I20–I25) morbidity	10,942	0.41 (0.05, 0.78)
				Other heart disease (ICD10 I30–I52) morbidity	3572	−0.26 (−0.81, 0.28)
				Cerebrovascular disease (ICD10 I60–I69) morbidity	12,832	0.45 (0.11, 0.80)
				All circulatory disease (ICD10 I00–I99) morbidity	32,189	0.18 (−0.03, 0.39)
German uranium miner study	Kreuzer et al (2006)	0.041 (0–>0.3)	59,001 (1,801,626)	All circulatory disease (ICD10 I00–I99)	5417	−0.26 (−0.6, 0.05) [n]
				Heart disease (ICD10 I00–I52)	3719	−0.35 (−0.7, 0.009) [n]
				Cerebrovascular disease (ICD10 I60–I69)	1297	0.09 (−0.6, 0.8) [n]
BNFL workers	McGeoghegan et al (2008)	0.0569 (0–>0.729)	38,779 (1,081,570)	Ischaemic heart disease (ICD9 na)	4165 [b]	0.70 (0.37, 1.07) [b,k]
				Cerebrovascular disease (ICD9 430–438)	1365 [b]	0.66 (0.17, 1.27) [b,k]
				All circulatory disease (ICD9 390–438, 440–459)	6476 [b]	0.54 (0.30, 0.82) [b,k]
				Ischaemic heart disease (ICD9 na)	3567 [c]	0.70 (0.33, 1.11) [c,k]
				Cerebrovascular disease (ICD9 430–438)	1018 [c]	0.43 (−0.10, 1.12) [c,k]
				All circulatory disease (ICD9 390–438, 440–459)	5319 [c]	0.65 (0.36, 0.98) [c,k]
3rd Analysis of UK National Registry for Radiation Workers	Muirhead et al (2009)	0.0249 (<0.01–>0.4)	174,541 (3,900,000)	All circulatory disease (ICD9 390–459)	10,509	0.251 (−0.01, 0.54)
				Coronary heart disease (ICD9 410–414)	7168	0.259 (−0.05, 0.61)
				Circulatory disease not strongly related to smoking (ICD9 390–409, 415–440, 442–459)	2875	0.280 (−0.19, 0.85)
				Cerebrovascular disease (ICD9 430–438)	1817	0.161 (−0.42, 0.91)
US Oak Ridge workers	Richardson and Wing (1999)	na (0–>0.1)	14,095 (425,486)	Ischaemic heart disease (ICD8 410–414)	976	−2.86 (−6.90, 1.18)

TABLE 4.1 Continued

Data	Source	Average heart/brain dose (range) (Sv)	Numbers in cohort (person years follow-up)	Endpoint (mortality unless otherwise indicated)	Numbers of cases/deaths	ERR Sv^{-1} (with 95% CI)
IARC 15-country nuclear worker study	Vrijheid et al (2007)	0.0207 (0.0–>0.5)	275,312 (4,067,861)	Circulatory disease (ICD10 I00–I99, J60–J69, O88.2, R00–R02, R57)	8412	0.09 (−0.43, 0.70)
				Ischaemic heart disease (ICD10 I20–I25)	5821	−0.01 (−0.59, 0.69)
				Heart failure (ICD10 I50)	130	−0.03 (<0, 4.91)
				Deep vein thrombosis and pulmonary embolism (ICD10 I26, I60–I69, I80, I82)	104	−0.95 (−1.00, 9.09) [o]
				Cerebrovascular disease (ICD10 O88.2)	1224	0.88 (−0.67, 3.16)
				All other circulatory disease (ICD10 R00–R02, R57, I00–I99 excluding I20–26, I50, I60–69, I80, I82)	1133	0.29 (<0, 2.40)
Environmental studies						
Three Mile Island study	Talbott et al (2003)	0.0001 (0–>0.00016)	32,135 (561,063)	Heart disease (white males)	1079	−274 (−874, 438)
				Heart disease (white females)	1121	−951 (−1433, −390)

Notes
a Analysis based on colon dose.
b Analysis using underlying or contributing cause of death.
c Analysis using underlying cause of death.
d Analysis based on stomach dose, derived from Table 3 of Yamada et al (2004) with smoking and drinking in the stratification.
e Analysis excluding highest dose group (3.1–7.6 Gy).
f Based on brain dose.
g Based on heart dose.
h Based on lung dose.
i Based on carotid dose
j Numbers of persons in exposed and control groups.
k 90% CI.
l External gamma dose (Gy).
m Internal alpha dose (Sv), applying a radiation weighting factor of 1.
n Risk estimates in relation to cumulative whole body external gamma dose.
o Estimate derived from log-linear model, evaluated at 1 Sv.

The basis of all estimations of risk is the value of the excess relative risk (ERR) coefficient (ERR Gy^{-1}). As discussed in more detail in Section 4.9, for most medical studies the dose is from external low LET radiation so the unweighted absorbed organ dose (Gy) would be the same as the weighted equivalent (Sv) dose; in the atomic bomb survivor studies and many occupational groups there is a component of neutron (and other high LET) dose, and risks in the published studies risks are therefore generally quoted as per unit equivalent dose (Sv) – however, the component of high LET dose is generally small, so equivalent doses should be similar to the unweighted absorbed dose (Gy). In Tables 4.1–4.7 doses and risks are consistent with the published data, so in particular we use the measure of excess relative risk (ERR) per Sv (equivalent dose). Wherever possible this was taken directly from the relevant study. For certain studies (Davis et al, 1989; Talbott et al, 2003) this was estimated from tabulations of deaths by dose group in the various papers, together with zero-dose estimated deaths. To make such estimations, as in Little et al (2008, 2009a, 2010), a simple linear relative risk model was fitted by maximum likelihood, assuming Poissonian errors (McCullagh and Nelder, 1989), in which it was assumed that the expected number of deaths in dose group d with average organ dose D (in Sv) is given by

$$E_d \lambda (1 + \alpha D) \tag{1}$$

where E_d is the expected number of deaths in dose group d and λ is the multiplier of the expected number of deaths at zero dose. The parameter α is the ERR per Sv, and central (maximum likelihood) estimates and 95% profile likelihood confidence intervals (CI) (McCullagh and Nelder,1989) are given in Table 4.1. Model fits were performed using the EPICURE package (Preston et al, 1998).

For the peptic ulcer study (Carr et al, 2005) in which the most useful information given is estimates of the (adjusted) relative risk, RR_i (and associated CI) in each dose group i, estimates of α and associated CI are obtained by least squares, ie by minimising the sum of squares:

$$\Sigma_i \, w_i \, (RR_i - 1 - \alpha \, D_i)^2 \tag{2}$$

where w_i is the weight attached to dose group i, given by

$$w_i = 1/(CI_{ui} - CI_{li})^2 \tag{3}$$

Results are not presented for any cohort where the extra follow-up amounts to a year or less compared with another study that otherwise properly contains it; therefore we omit from further consideration the US nuclear worker study (Howe et al, 2004) which contains only one more year (1997) follow-up than the IARC 15-country study (Vrijheid et al, 2007) that otherwise subsumes it, and similarly the studies of Johnson et al (1999) and Atkinson et al (2004), both subsumed within the latest National Registry for Radiation Workers analysis cohort (Muirhead et al, 2009) and with final follow-up earlier than that of this larger group (13/12/1996 and 31/12/1997, respectively, compared with 31/12/2001).

4.4 Findings in the Japanese Atomic Bomb Survivors

Excess radiation-associated mortality due to heart disease and stroke has been observed in the LSS cohort – the Life Span Study of survivors of the atomic bombings in Japan (Table 4.1) (Shimizu et al, 2010). However, the shape of the dose–response was very uncertain, and there is no direct evidence of excess risk for either heart disease or stroke under 0.5 Sv (Shimizu et al, 2010). Statistically significant radiation-associated increases in mortality among the survivors were also found for digestive diseases and respiratory diseases (Preston et al, 2003). In the latest follow-up of the Adult Health Study (AHS) (a subcohort of the LSS subject to biennial assessments of morbidity), Yamada et al (2004) observed statistically significant radiation-associated excess risks for incidence of hypertension and myocardial infarction (Table 4.1). The study of Yamada et al (2004) was the only epidemiological study apart from those of Ivanov et al (2006) and of Azizova and Muirhead (2009) considered to assess morbidity rather than mortality. As can be seen from Table 4.2, there are excess mortality risks of certain specific forms of heart disease, in particular hypertensive heart disease, rheumatic heart disease and heart failure (Shimizu et al, 2010); however, a degree of caution is needed when looking at these detailed endpoints, since diagnostic accuracy may not be high for some of these diagnostic groups. In particular, 'rheumatic heart disease' on death certificates often was not rheumatic but was misdiagnosed as such by the certifying physician. Review of a number of clinical records showed that more than half the

TABLE 4.2 Japanese atomic bomb survivor circulatory disease excess relative risks, ERR per Sv, by endpoint (Shimizu et al 2010) (all using underlying cause of death only)

Disease category (ICD 9)	Deaths	p-value	ERR Sv^{-1} (with 95% CI)
Circulatory disease (390–459)	**19,054 (100%)**	**<0.001**	**0.11 (0.05, 0.17)**
Stroke (430–438)	**9,622 (50.5)**	**0.02**	**0.09 (0.01, 0.17)**
Cerebral infarction (433,434)	2,659 (14.0)	>0.5	0.04 (–0.10, 0.20)
Cerebral haemorrhage (431)	4,060 (21.3)	0.36	0.05 (–0.06, 0.17)
Subarachnoid haemorrhage (430)	461 (2.4)	0.09	0.30 (–0.04, 0.76)
Other or unspecified stroke	2,442 (12.8)	0.04	0.16 (0.01, 0.34)
Heart disease (390–398, 402, 404, 410–429)	**8,463 (44.4)**	**<0.001**	**0.14 (0.06, 0.23)**
Ischaemic heart disease (410–414)	3,252 (17.1)	>0.5	0.02 (–0.10, 0.15)
Myocardial infarction (410)	1,735 (9.1)	>0.5	0.00 (–015, 0.18)
Hypertensive heart disease (402, 404)	922 (4.8)	0.009	0.37 (0.08, 0.72)
Rheumatic heart disease (393–398)	242 (1.3) [a]	0.002 [a]	0.86 (0.25, 1.72) [a]
Heart failure (428)	2,983 (15.7)	0.003	0.22 (0.07, 0.39)
Other heart disease	1,064 (5.6)	>0.5	–0.01 (–0.21, 0.24)
Hypertensive disease without heart disease (401, 403, 405)	**411 (2.2)**	**>0.5**	**0.07 (–0.22, 0.55)**
Other circulatory disease (406–409, 439–459)	**558 (2.9)**	**>0.5**	**–0.01 (na, 0.34)**

a 'Rheumatic heart disease' on death certificates often was not rheumatic but was misdiagnosed as such by the certifying physician.

'rheumatic heart disease' death certificates were valvular or other disease, not associated with prior rheumatic fever (Dear et al, 1968).

A major strength of the atomic bomb survivor studies is availability of information on major lifestyle factors that modify circulatory disease risk. In particular, in the mortality study of Shimizu et al (2010) for a subsample of 36,468 survivors (out of the total cohort of 86,611) who were sent and responded to a postal questionnaire, information is available for sociodemographic factors (education and occupation), lifestyle factors (smoking and alcohol intake), and health variables (obesity and diabetes mellitus). Adjustment for all of these had little effect on the risks for any endpoint. In the morbidity study of Yamada et al (2004) information is available on smoking and alcohol intake. Again, adjustment for both of these had little effect on the risks for any endpoint.

4.5 Medium and Low Dose (Below 5 Gy) Therapeutically Exposed Groups

All the studies considered in this section are of patients treated for benign disease. In contrast to the high doses typical of treatment for malignant disease, doses are generally much lower (typically below 5 Gy) in most groups treated for benign disease (McGale and Darby, 2005). There was a significant (2-sided $p = 0.01$) increasing trend of coronary heart disease mortality with radiation dose in a US cohort of persons treated for peptic ulcer (half with X-irradiation, half without), although there was no such significant trend for other cardiovascular mortality (Carr et al, 2005). Doses in this study are among the highest considered here. In this study the mean heart dose was 1.3 Gy (range 0.0–7.6) (Carr et al, 2005) (Table 4.1). Of the 3043 patients in this study, 382 received average cardiac doses of 3.1–7.6 Gy, with a mean dose to the part of the heart in the beam of 18.4 Gy (range 14.4–35.6 Gy). This high dose group is excluded in estimating the ERR; as shown by Little et al (2008) excluding this highest dose group from the regressions has little effect on the central estimates, although confidence intervals become appreciably wider. There is significant excess risk of coronary heart disease for average heart doses above 2.6 Gy. Radiation-associated excess mortality from cardiovascular disease has not been seen in a study of UK ankylosing spondylitis patients (Darby et al, 1987), in which the mean heart dose was also high, 2.49 Gy (range 0–17.28 Gy), although the mean brain dose 0.14 Gy (range 0–4.80 Gy) (Lewis et al, 1988) is somewhat lower (Table 4.1). It should be noted that the study of Davis et al (1989) reported an overall standardised mortality ratio (SMR) and not a dose–response analysis. The risk that we cite, based on this SMR, might well be biased.

4.6 Diagnostically Exposed Groups

No excess circulatory disease mortality has been observed in a cohort of Massachusetts tuberculosis patients receiving multiple fluoroscopic chest X-rays (Davis et al, 1989), although the lung (and probably heart) dose in this group was fairly low, an average of 0.84 Gy (Table 4.1). Although not reported in this table, there have been a number of groups exposed to internally deposited radionuclides, in particular alpha particles from the diagnostic contrast medium Thorotrast. Among the largest of these is a cohort of US patients (Travis et al, 2001) which reported marginally significant elevations in risk from cardiac disease

[for males relative risk, RR = 1.0 (95% CI 0.8, 1.2), for females RR =1.2 (95% CI 1.0, 1.6), total RR = 1.1 (95% CI 0.9, 1.3)], although for cerebrovascular disease there were more substantial (and statistically significant) elevations [for males RR = 1.4 (95% CI 1.0, 2.0), for females RR =1.8 (95% CI 1.3, 2.5), total RR = 1.6 (95% CI 1.2, 2.0)]. In a somewhat smaller Portuguese series risks of circulatory disease were not significantly elevated [for males RR = 1.11 (95% CI 0.76, 1.62), for females RR = 0.97 (95% CI 0.53, 7.70), total RR = 1.08 (95% CI 0.79, 1.46)] (dos Santos Silva et al, 2003). The findings in relation to cerebrovascular disease in the US series should be treated with caution, since a frequent reason for use of Thorotrast was investigation of cerebral vascular anomalies, as pointed out by Travis et al (2001). Thorotrast deposits alpha-particle dose primarily to the liver. Unfortunately, to the best of our knowledge, evaluation of these health endpoints in relation to liver doses has not been performed.

4.7 Occupationally Exposed Groups

There are increasing trends for certain cardiovascular disease mortality endpoints (all circulatory disease, cerebrovascular disease and other circulatory diseases), and decreasing trends for certain other endpoints (ischaemic heart disease, heart failure, deep vein thrombosis and pulmonary embolism) in the IARC 15-country study of radiation workers (Vrijheid et al, 2007) (Table 4.1), although none is statistically significant (1-sided $p \geq 0.20$). Radiation-associated excess ischaemic heart disease and stroke morbidity has been observed in excess in a group of Chernobyl recovery workers, although there was no excess morbidity due to hypertensive heart disease and other heart disease (Ivanov et al, 2006) (Table 4.1). There is a very strong, and highly statistically significant, increasing trend of circulatory disease mortality with dose in a Canadian cohort of nuclear workers and various other occupationally exposed groups (dentists, radiographers, etc) (Ashmore et al, 1998) (Table 4.1). However, general increases of the same sort of order were seen for a number of other diseases in this study, which implies that there may be bias. A highly statistically significant trend with dose has been seen for ischaemic heart disease and cerebrovascular disease in the latest analysis of the Mayak worker data. As with the atomic bomb survivor data and the Chernobyl liquidator cohort of Ivanov et al (2006), this cohort is unusual in that information on morbidity and mortality is available, as well as information on smoking and alcohol consumption. The study is also unusual in that doses to certain internal organs, in particular the lung and liver, are dominated by doses from internally deposited radionuclides, in particular the alpha-emitting radionuclide plutonium. There is a significant dose–response in relation to both external gamma dose and internal (alpha-particle) dose to the liver (Table 4.1). There are few cohorts of any substance apart from this with alpha-particle liver dose. Groups exposed to the diagnostic contrast medium Thorotrast received a substantial alpha-particle liver dose, as discussed above, and it is notable that there is little evidence of excess risk of circulatory disease, specifically cardiac disease, in these cohorts.

A borderline significant trend with dose has been seen for circulatory disease in the latest analysis of the UK National Registry for Radiation Workers (Muirhead et al, 2009), an excess relative risk of 0.25 Sv^{-1} (95% CI −0.01, 0.54). An increasing trend with dose of a similar magnitude is also seen for all cancer mortality, an ERR of 0.28 Sv^{-1} (95% CI −0.02, 0.62). In other workforces (Richardson and Wing, 1999; Vrijheid et al, 2007) there are generally no statistically significant trends of circulatory disease with dose (Table 4.1). It should be noted that these studies overlap, and in particular there is substantial inclusion of

the study populations of the studies of Richardson and Wing (1999) and Muirhead et al (2009) with that of the IARC 15-country study (Vrijheid et al, 2007). There were no statistically significant trends of circulatory disease mortality with cumulative radon, external gamma or dose from other radionuclides in a cohort of male German uranium miners (Kreuzer et al, 2006) (Table 4.1); similar results were reported in a reanalysis of this cohort that added five more years of follow-up (1999–2003) (Kreuzer et al, 2010). There was also no trend with any measure of dose for ischaemic heart disease (Kreuzer et al, 2006), coronary heart disease or stroke (Kreuzer et al, 2010); mortality from acute myocardial infarction exhibited a borderline significant (2-sided p = 0.114) increasing trend with radon dose (Kreuzer et al, 2010), although the authors were inclined to treat this as spurious (Kreuzer et al, 2006). Heart doses both from radon and external gamma were low; the average gamma dose was 0.041 Sv, with only 124 workers receiving doses of 0.5 Sv or more. Despite the large number of deaths (5417) from circulatory disease, therefore, the statistical power of this study was low. There is no significant trend of coronary heart disease mortality with radon dose in a cohort of Canadian fluorspar miners (Villeneuve et al, 2007).

It is generally unclear from the published analyses at what dose the dose–response is conventionally statistically significant.

4.8 Environmentally Exposed Groups

There was a decreasing trend in heart disease mortality with dose for males and females in the study of Talbott et al (2003) of persons exposed as a result of the accident at the Three Mile Island nuclear power station. For females the decreasing trend was significant. The contrast with the conclusions reported by Talbott et al (2003) should be noted: we base our conclusions on trends of SMR with dose, whereas Talbott et al interpreted elevations in SMR at various dose levels as evidence for significant excess risk. As with all studies of environmental exposure, exposure assessment in this study is problematic, although an attempt has been made to assess individual residence and migration patterns. An additional complication in relation to assessing cardiovascular endpoints is that stress would be expected to be associated with proximity to the plant, and therefore with dose; this confounding would be expected to potentially positive bias the excess relative risk estimate. Given the very small estimated doses, and the possibility of bias, this study should be regarded as minimally informative.

4.9 Summary of Medium and Low Dose Epidemiological Studies

Although the aggregate estimate of risk (see the meta-analysis below – Tables 4.3, 4.4 and 4.5) in these studies is suggestive of a positive association, the obvious heterogeneity complicates any causal interpretation.

The variation in magnitude of trends of cardiovascular disease with dose, which spans at least two orders of magnitude (see Table 4.1), and the possibility of confounding and other sources of bias, mean that we cannot be sure that these statistical associations observed with radiation are causal in nature. The well-known independent risk factors for cardiovascular disease, such as cigarette smoking, diabetes, obesity, high blood pressure and high levels of blood LDL, were not available or not adjusted for in analyses of

TABLE 4.3 Ischaemic heart disease (ICD9 410–414) excess relative risks per Sv in various studies

Study	ERR Sv^{-1} (with 95% CI)
Atomic bomb morbidity (Yamada et al, 2004)	0.05 (−0.05, 0.16)
Mayak morbidity (Azizova and Muirhead, 2009, + Azizova et al, 2010)	0.11 (0.05, 0.17)
Chernobyl morbidity (Ivanov et al, 2006)	0.41 (0.05, 0.78)
Atomic bomb mortality (Shimizu et al, 2010)	0.02 (−0.10, 0.15) [a]
UK National Registry for Radiation Workers (Muirhead et al, 2009)	0.26 (−0.05, 0.61)
BNFL mortality (McGeoghegan et al, 2008)	0.70 (0.37, 1.07) [b,c]
US peptic ulcer mortality (Carr et al, 2005)	0.11 (0.01, 0.22)
UKAEA mortality (Atkinson et al, 2003)	−0.66 (−1.46, 0.23)
US Oak Ridge mortality (Richardson and Wing, 1999)	−2.86 (−6.90, 1.18)
All occupational studies	**0.12 (0.06, 0.18)**
All studies	**0.10 (0.05, 0.14)**

Notes
a Analysis using underlying cause of death.
b Analysis using underlying or contributing cause of death.
c 90% CI.

most of these study groups. Only the atomic bomb survivor studies of Yamada et al (2004) and Shimizu et al (2010) and the Mayak worker studies of Azizova and Muirhead (2009) and Azizova et al (2010) incorporate adequate adjustment for the major lifestyle risk factors (smoking and alcohol consumption); while the study of Shimizu et al (2010) goes much further than this, adjusting for education, occupation, obesity and diabetes mellitus. This is likely to be particularly problematic in cohorts in which there was no adjustment for socioeconomic status in the analysis [all except Howe et al (2004), Vrijheid et al (2007), McGeoghegan et al (2008) and Muirhead et al (2009)]; many of these risk factors, in particular obesity, shift work and cigarette smoking, are correlated with socioeconomic status, and socioeconomic status may well be associated with occupational radiation exposure.

Among those treated with radiotherapy for malignant conditions, patients treated for breast cancer show promise for risk estimation because of the substantial and variable heart doses. Although cardiac doses are steadily reducing over time for new treated patients, they still remain high for the most part (Darby et al, 2005b). For example, a survey of 32 patients using three different radiotherapy regime plans for treatment of internal mammary lymph nodes assessed mean cardiac dose to be around 4–8 Gy for tumours of the left breast, and 2–4 Gy for tumours of the right breast, with parts of the heart receiving more than 20 Gy in some patients from each group (Sautter-Bihl et al, 2002). However, certain other radiotherapy regimes give substantially lower doses (Taylor et al, 2007). For example, a reconstruction of doses given in the period from 1950 to 2000 to about 40 patients by left-sided radiotherapy of the supraclavicular fossa, or of the posterior axilla, estimated mean cardiac doses of about 0.3–0.8 Gy, with maximal doses of 0.7–1.4 Gy; right-sided radiotherapy delivers negligible cardiac dose (Taylor et al, 2007). To the best of our knowledge there is only a single study concentrating on cardiovascular disease subsequent to these lower dose radiotherapy procedures, although dosimetry was not reported (Woodward et al, 2006).

TABLE 4.4 Aggregate excess relative risks per Sv of circulatory disease in published medium and low dose (<5 Gy) epidemiological datasets with estimated average radiation dose to the heart and for which quantitative risk assessment is possible (using as endpoint mortality from circulatory disease unless otherwise indicated) (reproduced in part from Little et al, 2009a, 2010)

Description	Studies included	ERR Sv^{-1} (with 95% CI)
All occupational and environmental studies including McGeoghegan et al (2008) but excluding Muirhead et al (2009)	Talbott et al (2003)[a], Ivanov et al (2006), Kreuzer et al (2006), Vrijheid et al (2007), McGeoghegan et al (2008)[c], Azizova and Muirhead (2009) + Azizova et al (2010)[b]	0.19 (0.14, 0.24)**
All occupational and environmental studies excluding McGeoghegan et al (2008) but including Muirhead et al (2009)	Talbott et al (2003)[a], Ivanov et al (2006), Kreuzer et al (2006), Vrijheid et al (2007), Azizova and Muirhead (2009) + Azizova et al (2010)[b], Muirhead et al (2009)	0.19 (0.14, 0.23)**
Atomic bomb survivor and medical irradiation studies	Darby et al (1987)[d], Davis et al (1989), Yamada et al (2004)[f], Carr et al (2005)[g], Shimizu et al (2010)[e]	0.06 (0.03, 0.09)*
All studies including McGeoghegan et al (2008) but excluding Muirhead et al (2009)	Darby et al (1987)[d], Davis et al (1989), Talbott et al (2003)[a], Yamada et al (2004)[f], Carr et al (2005)[g], Ivanov et al (2006), Kreuzer et al (2006), Vrijheid et al (2007) , McGeoghegan et al (2008)[c], Azizova and Muirhead (2009) + Azizova et al (2010)[b], Shimizu et al (2010) [e]	0.09 (0.07, 0.12)**
All studies excluding McGeoghegan et al (2008) but including Muirhead et al (2009)	Darby et al (1987)[d], Davis et al (1989), Talbott et al (2003)[a], Yamada et al (2004)[f], Carr et al (2005)[g], Ivanov et al (2006), Kreuzer et al (2006), Vrijheid et al (2007), Azizova and Muirhead (2009) + Azizova et al (2010)[b], Muirhead et al (2009), Shimizu et al (2010)[e]	0.09 (0.07, 0.12)**

Notes
a Analysis based on heart disease (males and females separately).
b Analysis based on ischaemic heart disease and cerobrovascular disease morbidity, using external gamma dose.
c Analysis including underlying and contributory causes of death.
d Analysis based on stroke and other circulatory disease (separately).
e Analysis based on all circulatory disease, using underlying or contributing cause of death.
f Analysis based on morbidity from hypertension, hypertensive heart disease, ischaemic heart disease stroke and aortic aneurysm (separately).
g Analysis based on coronary heart disease and other heart disease, excluding highest dose group (3.1–7.6 Gy) (separately).
* p-value for heterogeneity $p < 0.0005$
** p-value for heterogeneity $p < 0.00000001$

The issue of publication bias is a problem for this review as for any such review and in particular previous such surveys (McGale and Darby, 2005; Little et al, 2008, 2009a, 2010). However, as radiation-induced cardiovascular disease has been an issue even in the LSS data for at least 15 years (Shimizu et al, 1992; Wong et al, 1993), arguably this should not greatly affect the findings of the systematic reviews of Little et al (2008, 2009a, 2010), and therefore the current document, concentrating as they do on results published since 1990.

TABLE 4.5 Sensitivity of combined circulatory disease risk estimates to study exclusion (excess relative risk per Sv) [all assuming as baseline inclusion of Muirhead et al (2009) and exclusion of McGeoghegan et al (2008) (as in the bottom row of Table 4.4)] and contribution to heterogeneity statistic (plus degrees of freedom, df)

Studies excluded	ERR Sv^{-1} (with 95% CI)	χ^2 (df)
Atomic bomb survivors (Yamada et al, 2004; Shimizu et al, 2010[a])	0.11 (0.07, 0.14)**	13.22 (6)
US peptic ulcer (Carr et al, 2005)	0.09 (0.07, 0.12)**	2.29 (2)
UK ankylosing spondylitis (Darby et al, 1987)	0.10 (0.07, 0.12)**	6.49 (2)
Massachusetts TB (Davis et al, 1989)	0.11 (0.08, 0.13)**	17.53 (1)
IARC 15-country nuclear workers (Vrijheid et al, 2007)	0.09 (0.07, 0.12)**	0.00 (1)
UK 3rd NRRW analysis (Muirhead et al, 2009)	0.09 (0.07, 0.12)**	1.17 (1)
German uranium miners (Kreuzer et al, 2006)	0.09 (0.07, 0.12)**	4.95 (1)
Chernobyl recovery workers (Ivanov et al, 2006)	0.09 (0.07, 0.12)**	0.68 (1)
Mayak workers (Azizova and Muirhead, 2009, + Azizova et al, 2010)	0.06 (0.03, 0.09)*	50.50 (2)
Three Mile Island (Talbott et al, 2003)	0.09 (0.07, 0.12)**	11.62 (2)
None (all studies)	**0.09 (0.07, 0.12)****	**108.44 (18)**

a Analysis including underlying and contributory causes of death.
* p-value for heterogeneity $p < 0.00001$
** p-value for heterogeneity $p < 0.00000001$

Radiation dosimetry is another issue that must be considered, particularly in relation to the radiotherapy studies. Related to this is the question of what the target tissue might be. There are indications that the immune system may be adversely affected in the Japanese atomic bomb survivors, suggested by variations of T-cell and B-cell population numbers with radiation dose (Kusunoki et al, 1998). Taken together with the known involvement of the immune system in cardiovascular disease, this implies that whole body dose (or possibly bone marrow dose) might be the most relevant dose. Of relevance are the diverse measures of dose used. In most of the studies considered here the predominant dose is from penetrating low LET radiation. In the Japanese atomic bomb survivors the dose used was DS02* colon (Shimizu et al, 2010) or DS86 stomach (Yamada et al, 2004) equivalent dose, with a radiation weighting factor (w_R) of ten for the small neutron component. Since there are only minor differences between organ doses for a particular survivor, even between the superficial and deep-lying organs, the error introduced by use of the colon or stomach as a surrogate for the heart or brain is probably small.

* DS02 and DS86 refer to standard systems for assessing the doses of those in the atomic bomb survivor cohorts.

In the peptic ulcer (Carr et al, 2005) and ankylosing spondylitis studies (Darby et al, 1987; Lewis et al, 1988) the dose used was that to the relevant tissue (heart or brain). In the TB fluoroscopy study (Davis et al, 1989) lung dose was used as a surrogate; heart dose would be expected to be similar to lung dose for the penetrating X-rays used here.

In the various occupational studies the dose used was generally (eg Vrijheid et al, 2007; McGeoghegan et al, 2008; Muirhead et al, 2009) external film badge dose, with appropriate radiation weighting factors for neutron radiation. For the German uranium miner study (Kreuzer et al, 2006) the doses from gamma radiation (and radon progeny, which are not considered here) were estimated via a job-exposure matrix. Dose from internal emitters was generally not taken into account in these studies, the significant exceptions being the Mayak workers study (Azizova and Muirhead, 2009), for which liver doses from plutonium were estimated, and the Canadian nuclear and other workers study (Ashmore et al, 1998), in which tritium doses were estimated. For most of these cohorts heart or brain doses from this source would not be expected to be significant compared to external gamma doses, although for the Mayak workers clearly the internal dose dominates for many organs (eg lung and liver). Similarly, the neutron dose in most studies was generally small relative to the low LET component, so that unless the radiation weighting factor were to be much greater than ten this should not affect dose estimates greatly.

It should be noted that in most of the studies, in particular those of the atomic bomb survivors and all occupational groups (given in Tables 4.1, 4.4 and 4.5), conventional ICRP radiation weighting factors are used, with the exception of the risks for the Mayak workers in relation to alpha-particle dose to the liver, for which a radiation weighting factor of one was used (Table 4.1). For this reason risks are given as per Sv equivalent dose rather than per Gy absorbed dose. In practice use of absorbed dose (Gy) rather than equivalent dose (Sv) would make little difference. The only cohort in which there was a substantial contribution from high LET radiation is that of the Mayak workers (Azizova and Muirhead, 2009). This cohort was analysed in relation both to external gamma dose and to internal alpha-particle dose to the liver. The heterogeneity in dose estimation criteria introduced by the dosimetry is therefore expected to be relatively minor, and would probably not contribute materially to the inconsistency in trends of dose–response relationships.

4.10 Meta-analysis of Available Epidemiological Studies

In Tables 4.3–4.6 we compute an aggregate estimate of excess relative risk across various of these studies using standard statistical methodology. The studies used are given in the leftmost column of Tables 4.3–4.5 and the second column of Table 4.6, and we justify the choice of these more carefully below. For those studies for which an ERR estimate together with a measure of standard deviation is available, we can compute the best linear unbiased estimate (inverse-variance weighted) of the ERR, given by

$$ERR_{tot} = \sum_i ERR_i / sd(ERR_i)^2 \Big/ \sum_i 1/sd(ERR_i)^2 \qquad (4)$$

This has standard deviation given by

$$\mathrm{sd}(\mathrm{ERR}_{\mathrm{tot}}) = 1 \Big/ \left[\Sigma_i \; 1/\mathrm{sd}(\mathrm{ERR}_i)^2 \right]^{0.5} \qquad (5)$$

These formulae are used to compute aggregate measures of the ERR and associated confidence intervals [obtained as $\mathrm{ERR}_{\mathrm{tot}} \pm 1.96 \times \mathrm{sd}(\mathrm{ERR}_{\mathrm{tot}})$] in Tables 4.3–4.6. It should be noted that equation 5 is an exact estimate of the standard deviation. However, when the component distributions are very markedly non-normal (eg if they are markedly asymmetrical), the resultant scaled linear sum (equation 4) will also be non-normal (eg asymmetric) in general. However, as can be seen from Table 4.1, most estimates of the ERR have approximately symmetrical confidence intervals about the mean, so it is expected that the scaled sum (equation 4) will also be approximately symmetrical about its mean. The standard deviations in the individual studies are estimated from the confidence intervals given in the published papers – for example, when the 95% CI is given, this is calculated from $(\mathrm{ERR}_{97.5} - \mathrm{ERR}_{2.5})/(2 \times 1.96)$. In many cases these confidence intervals appear to be derived from likelihood-based methods (McCullagh and Nelder, 1989), and this introduces additional approximation. We apply this formula to a subset of the studies in Table 4.1, selected so as to be more or less disjoint. [For example, we do not include the studies of Ashmore et al (1998) and Richardson and Wing (1999), since these are largely subsumed in the IARC 15-country study of Vrijheid et al (2007). Similarly we do not include the study of McGeoghegan et al (2008) whenever we include that of Muirhead et al (2009). However, the study of McGeoghegan et al, although partly subsumed in that of Muirhead et al, includes four more years of follow-up (2002–05), so we choose to consider it instead of the latter, at least for the purposes of certain analyses presented in Table 4.4.] Heterogeneity was assessed via the standard χ^2 statistic, which was calculated by

$$\chi^2 = \Sigma_i \left[(\mathrm{ERR}_i - \mathrm{ERR}_{\mathrm{tot}}) \Big/ \mathrm{sd}(\mathrm{ERR}_i) \right]^2 \qquad (6)$$

the significance of which was assessed via comparison against centiles of the χ^2 distribution with the relevant number of degrees of freedom (number of component risk estimates – 1).

The results of Table 4.4 suggest that the aggregate estimate of the ERR from all medium and low dose studies excluding the study of McGeoghegan et al (2008) but including the study of Muirhead et al (2009) is 0.09 Sv^{-1} (95% CI 0.07, 0.12); almost no difference is made by instead including McGeoghegan et al but excluding Muirhead et al – the aggregate estimate of the ERR is 0.09 Sv^{-1} (95% CI 0.07, 0.12). There is significant heterogeneity ($p < 0.01$) in risk between studies, among all groups considered in Table 4.4. Further analysis in which each study is removed in turn from the 'All studies excluding McGeoghegan et al' group in Table 4.5 does not substantially alter the aggregate risk estimate, which increased to at most 0.11 Sv^{-1} (95% CI 0.07, 0.14) (after exclusion of the Japanese atomic bomb survivor data), although removing the Mayak worker study of Azizova and Muirhead (2009) results in a substantial decrease in the best estimate of the ERR, to 0.06 Sv^{-1} (95% CI 0.03, 0.09). The results of Table 4.6 suggest that the heterogeneity between studies is not much diminished when the different endpoints (heart disease and stroke) are considered. However, the ERR for stroke is substantially higher 0.21 Sv^{-1} (95% CI 0.16, 0.27) than that for heart disease, 0.09 Sv^{-1} (95% CI 0.05, 0.12). Looking in more detail at

the risks for the most common form of heart disease, ischaemic heart disease, the results of Table 4.3 suggest that both for all occupational studies and for all studies altogether, there are highly significant excess risks – an ERR Sv^{-1} of 0.12 (95% CI 0.06, 0.18) and 0.10 (95% CI 0.05, 0.14), respectively. The morbidity risk of circulatory disease appears to be slightly higher, 0.10 Sv^{-1} (95% CI 0.07, 0.13) than that for mortality, 0.08 Sv^{-1} (95% CI 0.04, 0.12).

TABLE 4.6 Aggregate excess relative risks per Sv of circulatory disease by endpoint

Endpoint	Studies included	ERR Sv^{-1} (with 95% CI)	Heterogeneity
Heart	Darby et al (1987)[a], Talbott et al (2003)[b], Yamada et al (2004)[c], Carr et al (2005)[d], Ivanov et al (2006)[e], Kreuzer et al (2006)[b], Vrijheid et al (2007)[a], Azizova and Muirhead (2009) + Azizova et al (2010)[f], Shimizu et al (2010)[b]	0.09 (0.05, 0.12)	p = 0.0002
Stroke	Darby et al (1987)[g], Yamada et al (2004)[h], Ivanov et al (2006)[h], Kreuzer et al (2006)[g], Vrijheid et al (2007)[g], Azizova and Muirhead (2009)[h], Muirhead et al (2009)[g], Shimizu et al (2010)[g,l]	0.21 (0.16, 0.27)	p = 0.000002
Morbidity	Yamada et al (2004)[c h j], Ivanov et al (2006)[k], Azizova and Muirhead (2009) + Azizova et al (2010)[f h]	0.10 (0.07, 0.13)	$p < 10^{-9}$
Mortality	Darby et al (1987)[a g], Davis et al (1989)[i], Talbott et al (2003)[b], Carr et al (2005)[d], Kreuzer et al (2006)[i], Vrijheid et al (2007)[i], Muirhead et al (2009)[i], Shimizu et al (2010)[i,l]	0.08 (0.04, 0.12)	p = 0.0000005
Total	Darby et al (1987)[a g], Davis et al (1989)[i], Talbott et al (2003)[b], Yamada et al (2004)[c h j], Carr et al (2005)[d], Ivanov et al (2006)[k], Kreuzer et al (2006)[i], Vrijheid et al (2007)[i], Azizova and Muirhead (2009) + Azizova et al (2010)[f h], Muirhead et al (2009)[i], Shimizu et al (2010)[i,l]	0.09 (0.07, 0.12)	$p < 10^{-14}$

Notes
a Analysis based on all circulatory disease mortality apart from stroke.
b Analysis based on all heart disease mortality.
c Analysis based on morbidity from hypertensive heart disease, ischaemic heart disease and aortic aneurysm.
d Analysis based on coronary heart disease and other heart disease mortality, excluding highest dose group (3.1–7.6 Gy).
e Analysis based on morbidity from hypertension, ischaemic heart disease and other heart disease.
f Analysis based on morbidity from ischaemic heart disease.
g Analysis based on stroke mortality.
h Analysis based on stroke morbidity.
i Analysis based on all circulatory disease mortality.
j Analysis based on hypertension morbidity.
k Analysis based on all circulatory disease morbidity.
l Analysis including underlying and contributory causes of death.

4.11 Normalisations of Dose to Single Dose Equivalents and Implications for Heterogeneity in Cardiovascular Risk in the Atomic Bomb Survivor and Medium Dose Radiotherapy Studies

Schultz-Hector and Trott (2007) imply that by evaluating single dose equivalents of fractionation regimes used in certain of the radiotherapy studies that they considered, risks in these studies would become more compatible with those in the Life Span Study. In this section this question is briefly explored.

4.11.1 Dose normalisations

We calculate for the study of Carr et al (2005), one of those cited in Figure 1 of Schultz-Hector and Trott (2007), the physical single acute absorbed dose D' that would have equivalent biological effect to n equal fractions (each of D/n Gy) with total absorbed dose D Gy, assuming a total number of n = 16.5 Gy/1.5 Gy = 11 (see page 843 of Carr et al), for the range of total whole heart doses, D, given in Table 3 of Carr et al. We assume a linear-quadratic dose–response function, $f_D = C + \alpha D + \beta D^2$.

In this case D' is determined by

$$n[\alpha(D/n) + \beta(D/n)^2] = \alpha D' + \beta D'^2 \tag{7}$$

in other words by

$$D' = \frac{[D + (\beta/\alpha)\, D^2/n]}{\left(1 + \{1 + 4\,(\beta/\alpha)\,[D + (\beta/\alpha)\, D^2/n]\}^{0.5}\right)/2} \tag{8}$$

It should be noted that in the limit of a large number of fractions ($n \to \infty$), formula 8 tends to

$$D' = \frac{D}{\left(1 + \{1 + 4\,(\beta/\alpha)\, D\}^{0.5}\right)/2} \tag{9}$$

which in the low dose limit ($D \to 0$) becomes $D' = D$. This function is plotted in Figure 4.1 for the two values of α/β given above. Even at relatively high doses, of about 1 Gy, and assuming a low α/β of 1 Gy, the correction (expression 9) implies a reduction of no more than 40%.

4.11.2 Implications for cardiovascular risk in certain medical studies

We estimate the excess relative risks for various low and medium dose medical studies given in the systematic reviews of Little et al (2008, 2009a, 2010), with and without the acute dose corrections provided by expressions 8 and 9. To give an upper bound on the magnitude of the correction, we use the smaller of the two α/β ratios employed above, of 1 Gy (Lauk et al, 1987). However, we also use the central adjustment employed by Schultz-Hector and Trott, an α/β ratio of 2 Gy. We employ an estimated

eight fractions (4 Gy/0.5 Gy) derived from Trott and Kamrad (1999) for the UK ankylosing spondylitis data, and assume the large fraction approximation (expression 9) for the study of Davis et al (1989). The methodology used to estimate aggregate risk and estimate heterogeneity is as described above. As can be seen from Table 4.7, not much difference is made to the aggregate risk (radiotherapy plus atomic bomb) by use of this correction – the ERR changes from 0.06 Sv^{-1} (95% CI 0.03, 0.09) without correction to 0.07 Sv^{-1} (95% CI 0.04, 0.10) with an α/β ratio of 2 Gy, to 0.07 Sv^{-1} (95% CI 0.04, 0.10) with an α/β ratio of 1 Gy, although the significance of the heterogeneity is modestly reduced when corrections are made.

In summary, using a reasonable range of adjustments similar to those of Schultz-Hector and Trott (2007) we can reduce, but not eliminate, much of the difference in risk between the atomic bomb survivors and the radiotherapy studies.

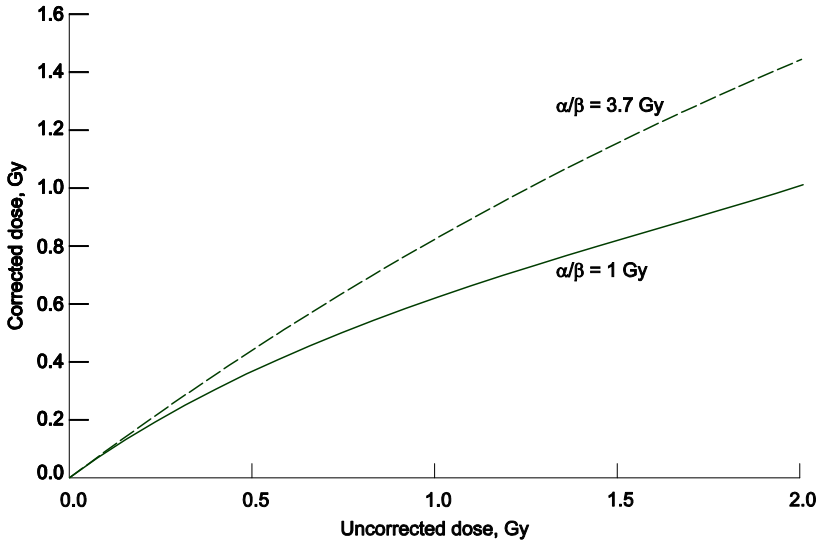


FIGURE 4.1 Corrected dose evaluated using expression 9 versus uncorrected dose

TABLE 4.7 Excess relative risks per Sv of cardiovascular disease in published medium and low dose (<5 Gy) A-bomb and medical datasets with estimated average radiation dose to the heart and for which quantitative risk assessment is possible (reproduced in part from Little et al, 2008, 2009a, 2010)

Data	Source	Average heart/brain dose (range) (Sv)	Endpoint (mortality unless otherwise indicated)	Unadjusted ERR Sv^{-1} (with 95% CI)	Acute adjusted ERR Sv^{-1} (with 95% CI) (α/β = 2 Gy)	Acute adjusted ERR Sv^{-1} (with 95% CI) (α/β = 1 Gy)
Japanese atomic bomb survivors						
Mortality	Shimizu et al (2010)	0.1 (0–4) [a]	Heart disease (ICD9 393–429 excluding 401, 403, 405)	0.18 (0.11, 0.25) [a]	–	–
			Stroke (ICD9 430–438)	0.12 (0.05, 0.19) [a]	–	–
			Circulatory disease apart from heart disease and stroke (ICD9 390–392, 401, 403, 405, 439–459)	0.58 (0.45, 0.72) [a]		
			All circulatory disease (ICD9 390–459)	0.15 (0.10, 0.20) [a]		
Morbidity	Yamada et al (2004)	0.1 (0–4) [b]	Hypertension incidence, 1958–1998 (ICD9 401)	0.05 (–0.01, 0.10) [b]	–	–
			Hypertensive heart disease incidence, 1958–1998 (ICD9 402, 404)	–0.01 (–0.09, 0.09) [b]	–	–
			Ischaemic heart disease incidence, 1958–1998 (ICD9 410–414)	0.05 (–0.05, 0.16) [b]	–	–
			Myocardial infarction incidence, 1964–1998 (ICD9 410)	0.12 (–0.16, 0.60) [b]	–	–
			Aortic aneurysm incidence, 1958–1998 (ICD9 441, 442)	0.02 (–0.22, 0.41) [b]		
			Stroke incidence, 1958–1998 (ICD9 430, 431, 433, 434, 436)	0.07 (–0.08, 0.24) [b]	–	–

TABLE 4.7 Continued

Data	Source	Average heart/brain dose (range) (Sv)	Endpoint (mortality unless otherwise indicated)	Unadjusted ERR Sv^{-1} (with 95% CI)	Acute adjusted ERR Sv^{-1} (with 95% CI) (α/β = 2 Gy)	Acute adjusted ERR Sv^{-1} (with 95% CI) (α/β = 1 Gy)
Low and medium dose radiotherapy and medical diagnostic studies						
Peptic ulcer study	Carr et al (2005)	1.3 (0.0–7.6)	Coronary heart disease (ICD8 410–414) [c]	0.10 (−0.12, 0.33)	0.15 (−0.21, 0.51)	0.17 (−0.24, 0.58)
			Other heart disease (ICD8 400–404, 420–429) [c]	−0.16 (−0.49, 0.17)	−0.25 (−0.76, 0.26)	−0.28 (−0.86, 0.30)
Ankylosing spondylitis	Darby et al (1987)	0.14 (0.0–4.80) [d]	Stroke (ICD7 430–434)	−2.43 (−4.29, 0.71) [d]	−2.57 (−4.53, 0.75) [d]	−2.68 (−4.74, 0.79) [d]
		2.49 (0.0–17.28) [e]	Other circulatory disease (ICD7 400–429, 435–468)	−0.01 (−0.12, 0.13) [e]	−0.02 (−0.18, 0.20) [e]	−0.02 (−0.20, 0.22) [e]
TB fluoroscopy	Davis et al (1989)	0.84 [f] (na)	All circulatory disease (ICD8 390–458)	−0.11 (−0.20, −0.01) [f]	−0.14 (−0.26, −0.01) [f]	−0.17 (−0.31, −0.02) [f]
Aggregate				**0.06 (0.03, 0.09)**	**0.07 (0.04, 0.10)**	**0.07 (0.04, 0.10)**
p-value for heterogeneity				**p = 0.000162**	**p = 0.000520**	**p = 0.000812**

Notes

a Analysis based on colon dose, using underlying or contributing cause of death.

b Analysis based on stomach dose, derived from Table 3 of Yamada et al (2004) with smoking and drinking in the stratification.

c Analysis excluding highest dose group (3.1–7.6 Gy).

d Based on brain dose.

e Based on heart dose.

f Based on lung dose.

4.12 Summary

1 There are a large number of radiotherapy studies showing a clear excess of circulatory disease risk; individual dosimetry has yet to be performed in most published studies, so they are of limited use for quantitative risk assessment.

2 There is accumulating epidemiological evidence for excess circulatory disease risk at moderate and low dose (below 5 Gy), in particular in the Japanese atomic bomb survivors and in various occupational studies (of nuclear workers).

3 Few studies (only those of the Japanese atomic bomb survivors and the Mayak nuclear workers) adequately control for major lifestyle and health factors (eg cigarette smoking and alcohol consumption).

4 There is statistically significant heterogeneity in risk among the informative medium and low dose studies, at least part of which is likely to be explained by confounding with unmeasured lifestyle factors in these groups. The heterogeneity is diminished (but not eliminated) if allowance is made for endpoint (mortality vs morbidity, heart disease vs stroke) and for dose fractionation effects in the medical studies. Taking all medium and low dose studies together, a small but highly statistically significant excess relative risk of 0.09 Sv^{-1} (95% CI 0.07, 0.12) is indicated.

5 For the atomic bomb survivors (Shimizu et al, 2010) there is significantly elevated circulatory disease risk above 0.5 Sv, and in some occupational studies [eg Ivanov et al (2006), McGeoghegan et al (2008) and Muirhead et al (2009)] there are significant (or borderline significant) dose–response trends over the dose range 0–0.5 Sv.

5 Radiobiology of Cardiovascular Injury

5.1 Pathogenesis and Endpoints for Radiation-induced Heart Damage

Many experimental animal studies of the effects of radiation on the cardiovascular system are available, largely from literature dating back over ten years. There are several reviews available (Schultz-Hector, 1992; Schultz-Hector and Trott, 2007; Hendry et al, 2008; Darby et al, 2010). As noted in Chapter 1, there are several types of cardiovascular injury caused by radiation, and the pathology of the main forms of heart damage are considered here.

5.1.1 Pericardial disease

Pericarditis is associated with oedema, fibrotic thickening and adhesions of the epicardium and pericardium, and is probably due to injury of the mesothelial cells. In rats, pericarditis was shown to be reversible after single doses of less than 20 Gy or equivalent absorbed doses of fractionated irradiation, but congestive heart failure was observed at dose-dependent latency times after recovery from pericarditis. After higher radiation doses, pericarditis was life threatening within 100–120 days (Lauk et al, 1985). However, this 'early' pericarditis was not evident in all strains of rat investigated (Yeung and Hopewell, 1985; Lauk, 1986). Not only the incidence, but also the time-course, of exudative pericarditis was found to be similar for different species. In dogs the risk of radiation-induced pericardial effusions reached a maximum at about three months after fractionated heart irradiation (McChesney et al, 1988). Further details of dose levels, latency times and fractionation effects are given in Section 5.2.

5.1.2 Cardiac functional injury

At later times after irradiation there may be decreased cardiac function, ischaemia, and fibrosis leading to heart failure. Myocardial degeneration, seen from about ten weeks after irradiation, coincided with the first signs of decreased cardiac function in rats (Schultz-Hector, 1992). However, further decreases in function did not occur until shortly before the onset of fatal congestive heart failure, despite continuing degeneration of myocardial mass. In rats total body irradiation of 10 Gy has been observed to lead to cardiac dysfunction at 120 days attributable to myocardial microvascular damage (Baker et al, 2009) and 20 Gy local irradiation of the rat heart can lead to cardiac dysfunction at 180 days (Benderitter et al, 1995). Cardiac performance has been assessed by an external counting technique for cardiac output (Yeung and Hopewell, 1985), and left ventricular ejection fraction as determined by ECG gated radionuclide ventriculography (Schultz-Hector et al, 1992a). Another assessment of cardiac performance after irradiation was the use of an *ex vivo* isolated working rat heart preparation (Wondergem et al,

1991). This showed more marked decreases in cardiac function with increasing times after irradiation compared to *in vivo* measurements (Schultz-Hector, 1992).

Local irradiation of rat hearts with single or fractionated doses leads to heart failure after dose-dependent latency times (Schultz-Hector et al, 1992b). The signs of heart failure were dyspnoea at rest, apathy and subcutaneous oedema. Animals autopsied when they presented with these symptoms had a congested liver and occasional pleural effusions. The left ventricle was dilated, showing a reduction in wall thickness by 15–17% of control values. Histological examination revealed a focal degeneration and necrosis of about 23% of the total myocardial volume. Loss of alkaline phosphatase activity from myocardial capillaries, which is known to precede myocardial degeneration, involved 77% of the myocardium. These findings at the time of manifest heart failure were constant, independent of whether injury to the heart was inflicted by single-dose or fractionated irradiation, or whether heart failure developed within a relatively short time after high or very high total doses or within many months after lower total doses. The latent time of heart failure therefore was considered an appropriate endpoint for comparison of treatment groups (Schultz-Hector et al, 1992b).

As described in earlier publications (Fajardo and Stewart, 1970; Lauk, 1987; Schultz-Hector, 1992; Fajardo et al, 2001), the earliest morphological changes seen in the irradiated heart are reversible changes in the function of capillary endothelial cells, leading to lymphocyte adhesion and extravasation. There is thrombus formation, obstruction of the microvessels and decrease in capillary density, accompanied by loss of the endothelial cell marker alkaline phosphatase, leading to focal interstitial fibrosis. Systematic morphometric studies in rats showed that capillary volume and length density began to decline about 20 days after heart irradiation, and the decline continued in a dose-dependent manner. When there was capillary rarefaction there was also a simultaneous focal loss of alkaline phosphatase (Schultz-Hector and Balz, 1994), which increased with time. Some evidence showed that this enzyme is involved in regulating endothelial cell proliferation and microvascular blood flow by dephosphorylating extracellular nucleoside phosphates. When foci of myocardial degeneration developed, they were invariably situated in areas of enzyme loss. Ultrastructural studies showed that the enzyme loss was not caused by a loss of endothelial cells but was associated with signs of endothelial cell activation, such as swelling, lymphocyte adhesion and extravasation (Schultz-Hector and Balz, 1994), as well as with increased endothelial cell proliferation (Lauk and Trott, 1990). Although the remaining capillary endothelial cells responded to damage by increased proliferation, this was inadequate to maintain proper microvascular function. Progressive reduction in the number of patent capillaries eventually led to ischaemia, myocardial cell death and fibrosis. Thus, the experimental animal studies point to radiation injury to the capillary network as the underlying cause of myocardial degeneration and heart failure after irradiation (Schultz-Hector and Trott, 2007). It should be noted nonetheless that damage to many cell types including cardiomyocytes and endothelial cells can contribute to cardiac injury and, furthermore, mast cell involvement cannot be excluded (Boerma et al, 2005, 2008).

Perfusion defects in the microvascular network of the heart have been demonstrated at the clinical level in breast cancer patients receiving radiotherapy and correlate with the experimental findings (Gallucci et al, 2008). Gyenes et al (1996) demonstrated perfusion defects, which localised to the irradiated left ventricle. Marks et al (2005) demonstrated that perfusion defects were progressive in terms of time and volume and were dependent on the volume of the myocardium exposed to the treatment field. Yu et al

(2004) demonstrated that some patients had persistent defects in perfusion and suggested that 'new' perfusion defects could develop three to four years after radiotherapy. These findings are in support of the experimental data, which indicate that damage to the microvascular network is progressive and suggest the underlying cause of ischaemic injury.

5.1.3 Radiation-induced atherosclerosis

Intimal thickening and perivascular fibrosis was associated with radiation-induced myocardial degeneration in dogs (McChesney et al, 1988). Normal rodents are known to be relatively resistant to arteriosclerosis from any cause (Ritskes-Hoitinga and Beynen, 1988), because of very low levels of low density lipoprotein (LDL) in their plasma. An example of radiation-induced ischaemic tissue atrophy in normal mice is atherosclerosis in the single central artery in the irradiated tail leading to tail atrophy and loss at various times more than five months post-irradiation (Hendry, 1987). Experimental studies on radiation-induced arteriosclerosis largely have focused on arteriosclerosis-prone animal models, which combine one or more risk factors with radiation. In spontaneously hypertensive rats, heart irradiation caused arteriosclerotic changes and arteriole obliterations as well as myocardial degeneration (Lauk and Trott, 1988). Hypertension was more pronounced in male than in female spontaneously hypertensive rats and was associated with shorter survival times after heart irradiation. Although antihypertensive treatment of male rats had no effect on survival after irradiation, a synergistic action of hypertension and heart irradiation was suggested by these findings.

Examples of radiation-induced fibrosis and atherosclerosis in human cardiovascular tissues are illustrated in Figures 5.1 and 5.2.

Studies in hypercholesterolemic rabbits and mice found that irradiating large arteries accelerated the formation of atherosclerotic plaque (Konings et al, 1978; Vos et al, 1983; Tribble et al, 1999; Cottin et al, 2001; Pakala et al, 2003). The initial event appeared to be endothelial cell damage, leading to monocyte adhesion and transmigration into the subendothelial space. In the presence of elevated cholesterol levels, these invading monocytes transformed into activated macrophages, which ingested lipids and formed fatty streaks in the intima (Vos et al, 1983). Hypercholesterolaemia had to be present at the time of irradiation for this process to occur. Wild-type mice fed a normal diet after irradiation or transferred to a high fat diet more than two weeks after irradiation did not develop fatty streak lesions (Tribble et al, 1999). Also, irradiation of the heart in several hundred rabbits failed to produce coronary heart disease (Fajardo, 1977; Stewart and Fajardo, 1978). A high fat diet was found to be necessary for irradiation to induce atherosclerosis (Amromin et al, 1964). However, plaques developed in dogs receiving a normal diet (Lindsay et al, 1962a,b). In general, results from these and other studies (Artom et al, 1965; Bradley et al, 1981) showed that the combination of irradiation and a high fat diet accelerated atherogenesis. The process was inhibited by using the anti-oxidant, CuZn-superoxide dismutase (Tribble et al, 1999). This suggested that irradiation initiates the formation of atherosclerosis by inducing oxidative damage in the vessels, which enhances oxidation of low density lipoproteins and allows them to be ingested by macrophages. General observations on atherogenesis suggest that smooth muscle cell activation, migration from the media, and proliferation within the lesion occur at a later stage.

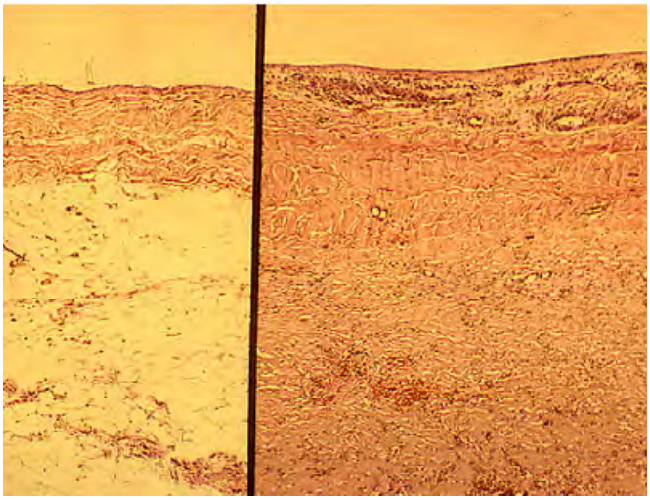


FIGURE 5.1 Perpendicular sections of human pericardium

The left panel shows the normal parietal pericardium with a thin, uniform fibrous layer that faces the heart (upper section) and an outer layer of adipose tissue (lower section). The right panel shows a typical example of irradiated pericardium at 17 months after receiving 67 Gy. The adipose tissue has been replaced by dense, fibrous tissue that actually extends well below the limits of the micrograph. Haematoxylin and eosin (HE) stain. (From Fajardo, 2005, with permission; described in Darby et al, 2010)

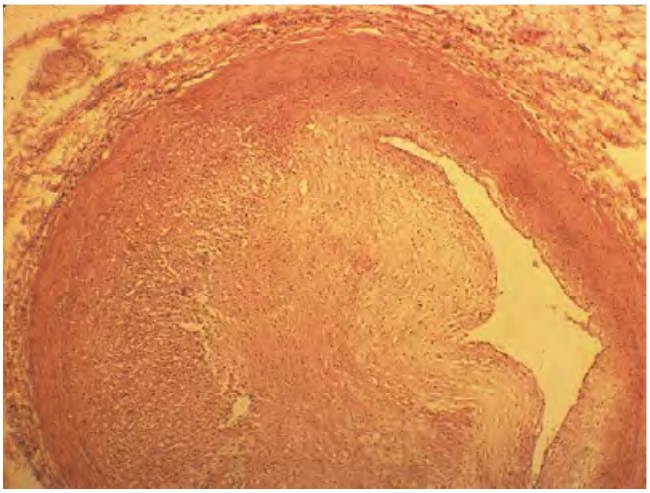


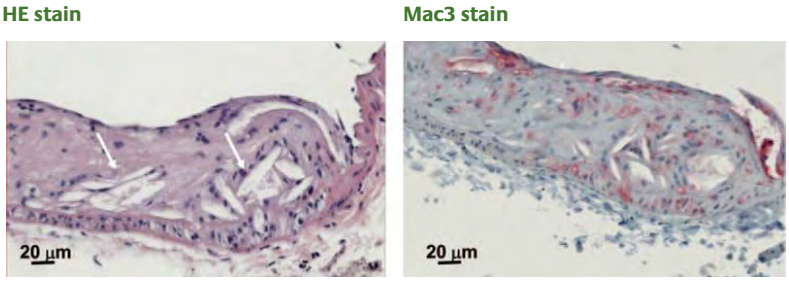
FIGURE 5.2 Left anterior descending coronary artery in a 16 year old boy, one year after receiving 40 Gy mantel radiotherapy for Hodgkin's disease

Myointimal proliferation has considerably narrowed the lumen. Fatal cases like this in a patient who had no cardiac risk factors other than radiation show that the morphology of arterial disease is essentially no different from that of age-related atherosclerosis. Haematoxylin and eosin (HE) stain. (From Fajardo, 2005, with permission; described in Darby et al, 2010)

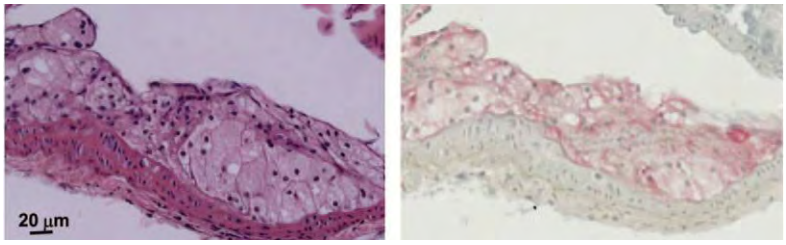
The carotid arteries of apolipoprotein-negative (ApoE-/-) mice were monitored for the development of atherosclerotic plaques for up to nine months after a single dose of 14 Gy (Stewart et al, 2006). Irradiation had no influence on cholesterol levels, markers of systemic inflammation, or atherosclerotic lesions in the non-irradiated renal arteries, but initial plaque formation began earlier and the rate of plaque growth was faster in irradiated than in non-irradiated carotid arteries. Histologically, the carotid arteries showed typical signs of plaque instability, such as intra-plaque haemorrhage and macrophage accumulation. The interaction of radiation-induced changes in endothelial function with the initial events of atherosclerotic lesion formation in these animals was believed to result in chronic inflammation, favouring the development of a vulnerable plaque. The lesions described are shown in Figure 5.3.

It was also investigated whether lower single doses and clinically relevant fractionated irradiation schedules predisposed to an inflammatory plaque phenotype (Hoving et al, 2008). ApoE-/- mice were given 8 or 14 Gy, or 20 x 2.0 Gy in four weeks to the neck, and the carotid arteries were subsequently examined for the presence of atherosclerotic lesions, plaque size and phenotype. At four weeks, early atherosclerotic lesions were found in 44% of the mice after single doses of 14 Gy but not in age-matched controls. At 22 to 30 weeks after irradiation there was a two-fold increase in the mean number of carotid lesions (8–14 Gy and 20 x 2.0 Gy) and total plaque burden (single doses only), compared with age-matched controls. The majority of lesions seen at 30 to 34 weeks after fractionated irradiation or a single dose of 14 Gy were granulocyte-rich (100% and 63%, respectively), with thrombotic features (90% and 88%), whereas these phenotypes were much less common in age-matched controls or after a single dose of 8 Gy. Hence it was shown that fractionated irradiation accelerated the development of atherosclerosis in ApoE-/- mice and predisposed to the formation of an inflammatory, thrombotic plaque phenotype.

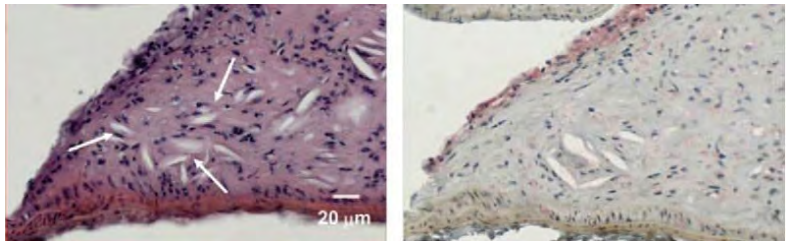
In addition to plaque formation, myointimal proliferation (ie of smooth muscle cells of the intima) in arteries leading to variable degrees of vessel occlusion may occur many months or even years after irradiation. A chance observation in a study designed to investigate the time sequence of changes in the spinal cord of pigs after irradiation, provided some insight into possible mechanisms of such arterial occlusions, albeit not in carotid arteries (reported by Sindermann et al, 2004). A single very high dose of 27.5 Gy cobalt-60 gamma rays was delivered to a 10 cm length of the cervical spinal cord in four month old female large white pigs. The observed major vessel changes were in the main ventral artery within the lining of the spinal cord. At the earliest time point, six weeks after irradiation, there was qualitative evidence for a reduction in the number of endothelial cells lining the wall of this blood vessel. From 10 to 12 weeks after exposure there had been the development of a clearly defined subendothelial space, which contained hyaline material (Figure 5.4A) and the infiltration of mononuclear cells from the blood. White blood cell adherence to the endothelium and an excess number of mononuclear cells in the lumen of the vessel were also noted, indicating chemo-attraction into the irradiated area (Figure 5.4B). By 12–14 weeks after irradiation the lumen of the vessel was reduced to a varying degree and the subendothelial space was filled by what appeared to be loose connective tissue (Figure 5.4C). At slightly later times (16–18 weeks) the subendothelial space was filled with dense tissue resulting in a varying degree of vessel occlusion (Figure 5.4D). The internal elastic lamina remained intact.



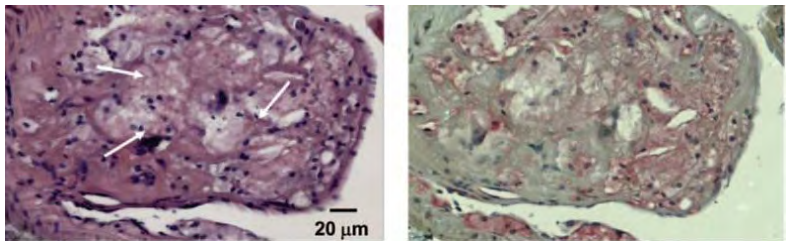
A Female mice at 22 weeks after 0 Gy (advanced lesion)



B Female mice at 22 weeks after 14 Gy (initial lesion)



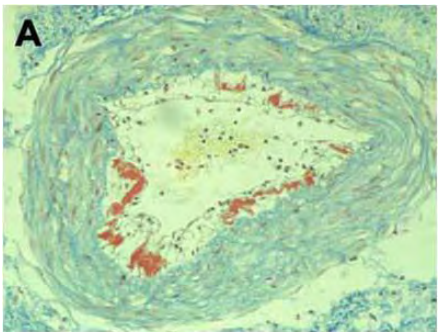
C Male mice at 28 weeks after 0 Gy (advanced lesion)



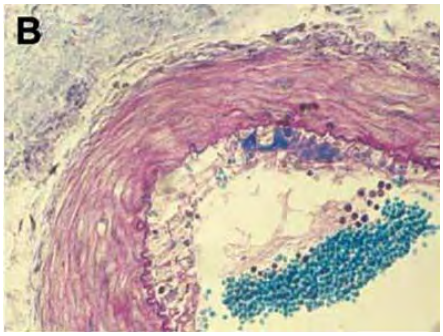
D Male mice at 28 weeks after 14 Gy (advanced lesion)

FIGURE 5.3 Inflammatory c phenotype of lesions in irradiated mouse carotid arteries

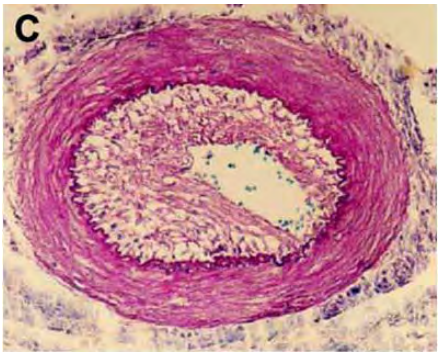
Representative photomicrographs showing haematoxylin and eosin (HE) staining in the left panels and Mac3 (110 kD type I membrane glycoprotein) staining of inflammatory cells (red colour) in the right panels. Sections are shown from carotid arteries of mice at times and doses stated. The arrows in A, C and D indicate necrotic cores in the advanced lesions. Photomicrographs were taken using a 40x objective and the scale bar is 20 μm. (From Stewart et al, 2006)



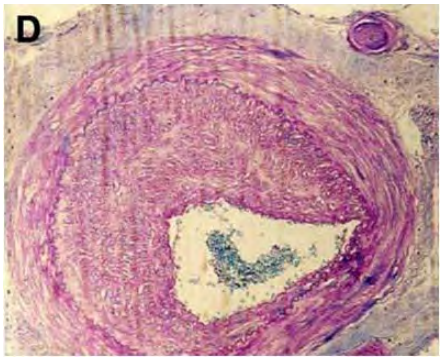
A 10 weeks, shows white blood cells accumulating in the lumen of the vessel and in the subendothelial space. The subendothelial space also shows an accumulation of hyaline material (MSB stain)



B Higher powered view of an adjacent section to show the subendothelial changes (Luxol fast blue, PAS)



C 14 weeks, shows almost complete occlusion of the vessel lumen with loose connective tissue (Luxol fast blue, PAS)



D 16 weeks, almost complete occlusion of the lumen of the blood vessel with dense connective tissue, and myointimal proliferation (Luxol fast blue, PAS)

FIGURE 5.4 Histological appearance of the inferior spinal artery of the spinal cord of pigs after the local irradiation of the cervical spinal cord with a single dose of 27.5 Gy cobalt-60 gamma rays

Histopathology courtesy of Professor J W Hopewell, published in Sindermann et al (2004)

In addition to the separate studies on macrovessels and microvessels, two hypotheses have been advanced for the biological mechanisms that lead to increased morbidity and mortality from coronary artery disease after radiation exposure in humans (Darby et al, 2010). The first hypothesis is that radiation increases the frequency of myocardial infarction by interacting with the pathological pathway of age-related coronary artery atherosclerosis, resulting in accelerated atherosclerosis in which disease is seen at a younger age than would normally occur. The second hypothesis is that radiation increases the lethality of age-related myocardial infarction by reducing the heart's tolerance to acute infarctions as a result of microvascular damage to the myocardium. These hypotheses are not mutually exclusive, and the two mechanisms may act together to produce clinical heart disease (Darby et al, 2010).

In man, the characteristic features of radiation-induced atherosclerosis include a more severe periarterial fibrosis and medial atrophy than with native disease, associated with severe intimal atherosclerotic disease, consisting of a necrotic core, fibrous tissue and calcification (Virmani et al, 1998, 1999). Radiation can also promote calcification of coronary arteries and valves in the absence of atherosclerosis in patients given radiotherapy as part of treatment for Hodgkin's disease (Apter et al, 2006), and occlusion of the vasa vasora has been found (Kalman et al, 1983). Indeed, the endothelium of the vasa vasora and the microvessels may be the structures most sensitive to radiation. Endothelial damage following irradiation of microvessel (capillaries, sinusoids and arterioles) was described over 100 years ago, with numerous subsequent reports of endothelial swelling, sloughing, fibrinoid necrosis, thrombus formation, and subsequent vessel fibrosis and occlusion (reviewed by Fajardo and Berthrong, 1988, and Basavaraju and Easterly, 2002). Similar changes have been described as necrotising vasculitis with significant changes in the vasa vasora, including swelling and detachment of the endothelium, subendothelial oedema, hyaline change and fibrinoid necrosis of the vessel walls with mononuclear cellular infiltration, accompanied by focal haemorrhages and chronic inflammation in the periadventitial soft tissue (Zidar et al, 1997).

Irradiation can induce a number of changes in normal arteries, including vascular smooth muscle cells (VSMC) and medial degeneration, intimal thickening, lipid deposition with occasional foam cell formation and adventitial fibrosis (Yang et al, 1978; Bradley et al, 1981; Yang and Ainsworth, 1982). All of these features are seen in human atherosclerosis. However, there is a long-standing literature that shows irradiation in a variety of experimental animals (rats, dogs, rabbits, pigeons and mice) generally accelerates the development of atherosclerosis in association with other risk factors, such as a high fat diet (Gold, 1962; Lindsay et al, 1962a,b; Lamberts and de Boer, 1963; Amromin et al, 1964; Artom et al, 1965; Sams, 1965; Kirkpatrick, 1967; Vesselinovitch and Wissler, 1968; Tiamson et al, 1970).

Case reports of patients with long survival times after local irradiation showing accelerated atherosclerosis indicate that radiation directly causes or promotes atherosclerosis in these circumstances. For example, lesions may occur at sites not normally involved in spontaneous atherosclerosis, with longer lesions containing more fibrous tissue (Loftus et al, 1987; Werner et al, 1988). Even without flow-limiting stenosis, local irradiation of the head and neck is associated with increased intima:media thickness, an early marker of atherosclerosis (Feehs et al, 1991; Cheng et al, 2002; Bilora et al, 2006). Following mediastinal irradiation, particular sites affected are those closest to the surface, including the coronary ostia and proximal left anterior descending artery (LAD), and also the internal thoracic arteries (Tracy et al, 1974; McEniery et al, 1987; Veeragandham and Goldin, 1998; Renner et al, 1999). Such focal lesions can be the cause of myocardial infarction and death in younger patients (Angelini et al, 1985), and widespread disease may promote ischaemic cardiomyopathy (Aronow et al, 1996). Similarly, involvement of head and neck vessels can promote stroke, and abdominal and pelvic vessels can produce small bowel infarction or limb ischaemia (Savlov et al, 1969; Nylander et al, 1978; Pettersson and Swedenborg, 1990; Patel et al, 2006). At its most catastrophic, radiation arteritis may induce vessel rupture (Fajardo and Lee, 1975).

Local endovascular brachytherapy from an internal, introduced source has been a successful treatment for coronary angioplasty and in-stent restenosis (King et al, 1998; Leon et al, 2001; Verin et al, 2001). The pathology and rationale for treatment of restenosis is, however, very different from that of atherosclerosis.

The restenosis lesion is due in part to proliferation of medial and intimal VSMCs at the site of injury, with subsequent deposition of extracellular matrix, and resolution of thrombus formation (Bennett and O'Sullivan, 2001). The purpose of brachytherapy is to induce a local, transient cessation of cell division in VSMCs that would otherwise contribute to the neointima formation. Whilst brachytherapy is effective in inhibiting proliferation, off-target effects have limited the use of this technology – for example, on cell division and function of endothelial cells with resultant impaired re-endothelialisation and late thrombosis (Virmani et al, 1998, 1999). The advent of drug-eluting stents has significantly reduced the use of brachytherapy, although in-stent restenosis is still a potential indication for its use.

In summary therefore, high doses of radiation (of the sort used in medical exposure, over 5 Gy) induce characteristic arterial damage in normal vessels, with some features similar to that seen in atherosclerosis. However, even medium dose irradiation accelerates primary atherosclerosis in association with other risk factors. High dose irradiation can reduce in-stent or angioplasty restenosis, although this lesion has a different pathology. A detailed discussion of the steps in the pathogenesis of atherosclerotic disease that may be affected by radiation exposures is given in Chapter 6, which also provides a schematic overview of the points at which radiation may act (see Figure 6.1).

5.2 Radiation Dose-latency and Dose–response Relationships

After single doses of 16 to 20 Gy to the heart, exudative pericarditis was shown to develop in rabbits, rats and dogs within 70–100 days (Fajardo and Stewart, 1970; Gavin and Gillette, 1982; Lauk et al, 1985; McChesney et al, 1988). The threshold dose was about 15 Gy, and the dose–response relationship was steep with the incidence rising to 100% at 20 Gy. An example in dogs after fractionated irradiation is shown in Figure 5.5.

In vitro, some effects of low to medium doses can be observed on endothelial cells. For example, E-selectin is an endothelial cell adhesion molecule mediating leukocyte rolling. E-selectin mRNA and protein were upregulated at two hours and four hours after a dose of 0.5 Gy (Hallahan et al, 1995, see Chapter 6). *In vivo*, there is a focal loss of histochemically detectable activity of endothelial marker enzymes such as alkaline phosphatase and 5'-nucleotidase, in parallel with alterations in capillary density. In distinct areas of the myocardium all capillaries had lost their enzyme activity, while endothelial cells appeared to be structurally intact in HE- or PAS-stained sections (Lauk, 1987). Enzyme loss always started prior to myocardial degeneration, and when foci of myocardial degeneration developed they were invariably situated within areas of enzyme loss. The maximum extent of enzyme-negative areas was dose dependent, amounting to 20% after 10 Gy, 60% after 15 Gy, and 90% of the myocardium after 20 Gy.

There were dose-related changes in the relative cardiac output of the rat heart at various time periods after 10–50 Gy single dose irradiation (Figure 5.6; Yeung and Hopewell, 1985). A single dose of 30 Gy reduced the cardiac output to about 70% of the control value, six months after irradiation.

The average survival time of rats after local heart irradiation (17.5–40 Gy) is shown in Figure 5.7, and survival times are dependent on the strain of rat used (Lauk, 1986).

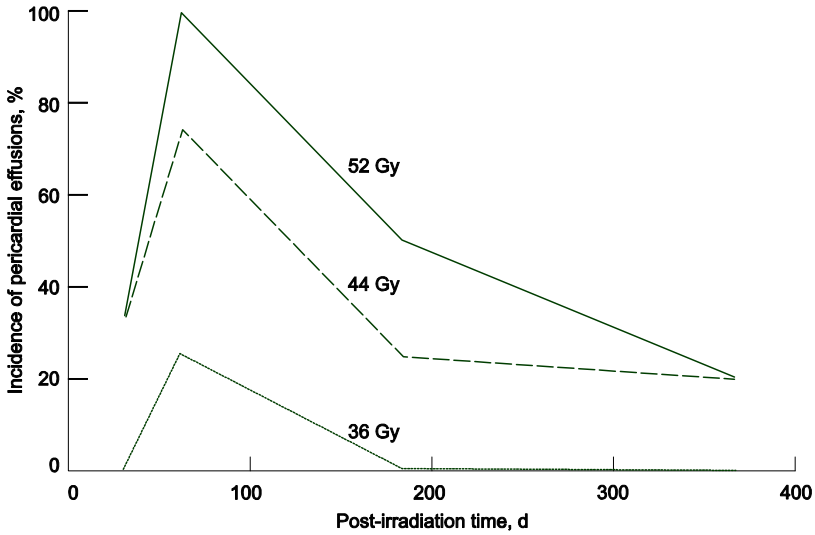


FIGURE 5.5 Variation of incidence of radiation-induced pericardial effusions in dogs with post-irradiation time. Pericardial effusions were measured at autopsy at 1, 3, 6 or 12 months. Fluid accumulations of more than 5 ml were considered to be abnormal effusions. Total doses of 36, 44 or 52 Gy had been given in 4 Gy fractions (McChesney et al, 1988; Schultz-Hector and Trott, 2007)

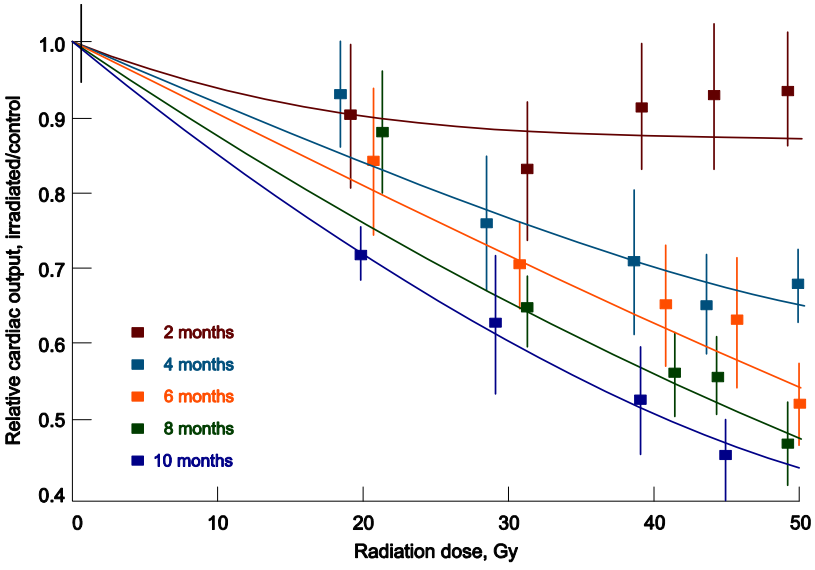


FIGURE 5.6 Dose-related changes in the relative cardiac output of the rat heart at various time periods after 10–50 Gy single dose irradiation. (From Yeung and Hopewell, 1985)

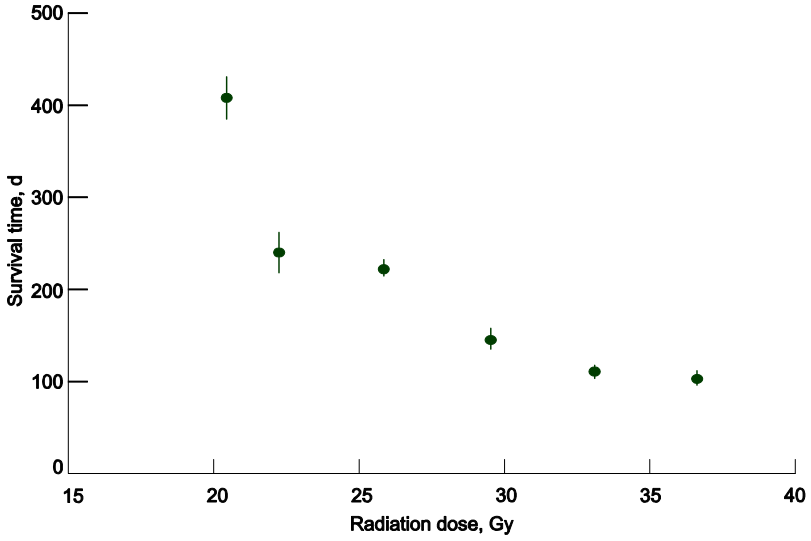


FIGURE 5.7 Average survival time (+/− SEM) of rats after local heart irradiation. (From Schultz-Hector and Trott, 2007)

5.3 Volume Effects

Mean radiation doses to the heart are usually quoted, but in many human situations the doses are very inhomogeneous across the heart, eg from breast cancer radiotherapy. The literature was reviewed recently to identify the main clinical and dose–volume predictors for acute and late radiation-induced heart disease (Gagliardi et al, 2010). It was concluded that a clear quantitative dose and/or volume dependence for most cardiac toxicities has not yet been shown, primarily because of the scarcity of the data. Several clinical factors, such as age, co-morbidities and the use of doxorubicin, appeared to increase the risk of injury.

There are as yet no animal studies that have investigated the effects of inhomogeneous dose distributions in the heart and the presence of any particularly sensitive regions of the heart.

The radiation response of the heart is influenced by radiation injury to the lung (reviewed by Schultz-Hector, 1992). Following irradiation of the rat heart, including 15% of the lung, a dose-dependent increase in breathing frequency was the earliest clinical symptom observed (Geist et al, 1990), although the right ventricular systolic pressure was not increased (Schultz-Hector, 1992). In dogs, McChesney et al (1991) systematically studied interactions of heart and lung irradiation. Three different radiation fields were chosen, encompassing either the heart including 36% of the lung, 80% of the lung excluding the heart, or the whole thorax. Giving a single dose of 12 Gy to the heart and to 36% of the lung resulted in a moderate increase of the pulmonary arterial pressure at six months. However, irradiation of the large lung field, excluding the heart, caused a much more pronounced pulmonary hypertension at six months, while whole thorax irradiation produced the same effect within three months. Thus severity of pulmonary hypertension appeared to be closely correlated to the amount of lung irradiated, independent of heart

irradiation. On the other hand, cardiac disease can be aggravated by additional lung damage, because cardiac stroke volume showed a moderate decrease after local heart irradiation, and a very significant decrease after whole thorax irradiation.

There is evidence that not only functional but also morphological changes in the myocardium can be severely aggravated by additional radiation-induced lung damage. Brown et al (1973) showed that local heart irradiation in C3H mice with doses as high as 40 Gy did not produce any clinical symptoms or significant myocytolysis within six months. The only obvious pathological findings were extensive mural thrombi. The authors concluded that the myocardium of mice is more resistant than in other species and that the presence of thrombi could be due to species variations in tissue levels of plasminogen activator. However, since no intravascular thrombi were described, arrhythmia and disturbance of cardiac contraction should be considered as an alternative explanation (Schultz-Hector et al, 1992a). On the other hand, whole thorax irradiation in B6CF1 mice with only 8 Gy caused pronounced myocytolysis on the ultrastructural level (Yang et al, 1976). The contradictory findings in these two studies could be due partly to mouse strain differences or to different sensitivities of the measurements (Schultz-Hector et al, 1992b).

5.4 Dose-time-fractionation Effects

From experiments giving one, two, four or ten dose fractions to the heart in rats, a low α/β ratio of 3.7 Gy (95% CI 1.8, 5.6) was calculated, using the latent time before heart failure as an endpoint (Schultz-Hector et al, 1992a). When the time interval between dose fractions was varied in a split-dose experiment, time intervals of up to three hours did not increase the survival time significantly, indicative of a very slow repair of sublethal radiation damage. The high fractionation sensitivity was also observed in dogs in earlier studies, where values of α/β ratios ranging from 2.6 Gy to 5.4 Gy were reported for various parameters including the myocardial connective tissue component, diastolic wall thickness and incidence of myocytolysis or ECG-changes (Gillette et al, 1985; McChesney et al, 1991). Split-dose studies with longer time intervals were performed by Wondergem et al (1996), using an *ex vivo* working rat heart preparation as a measure of cardiac function. The irradiated hearts were capable of repairing a large part of an initial single dose of 10 Gy within 24 hours. Thereafter the tolerance to a second dose decreased over many months, and the decrease was greater after higher initial doses.

5.5 Summary

1 In animal models it has been shown that early radiation-induced pericarditis is reversible after a single dose of less than 20 Gy.

2 Cardiac function in animal models declines after a dose-dependent latency period (a single dose of more than 20 Gy, a period of over four months).

3 Radiation predisposes to the formation of an inflammatory, thrombotic plaque phenotype in arteries.

4 The presence of any degree of genetic predisposition to atherosclerosis is unclear.

5 Oxidative damage is implicated in atherosclerosis, supported by experimental studies in ApoE-/- rodents.

6 A radiation-induced persistent decrease in capillary density is associated with focal loss of alkaline phosphatase.

7 Vessel occlusion can occur many years after irradiation, and the precise mechanisms of this are not fully known.

8 The heart in rodents shows a high sparing effect of radiation dose fractionation, as do other late-reacting organs, and evidence in humans is consistent with this effect.

9 Doses to the heart are often inhomogeneous; generally mean doses are quoted, and the critical heart structures pertaining to heart radiosensitivity are, as yet, unknown.

6 Potential Mechanisms of Cardiovascular Injury and Their Relevance to Radiation

The pathological consequences of radiation exposure have been well documented in Chapter 5. Here, we will now describe the possible mechanisms, at a cellular, subcellular and molecular level, by which radiation exposure may cause circulatory damage. Importantly, many of the effects of radiation, including:

a DNA damage,

b Mutation and cell transformation,

c Cell proliferation and senescence,

d Cell death,

e Mitochondrial dysfunction,

f Thrombosis,

g Inflammation,

h Fibrosis,

are also implicated in the mechanism for cardiovascular disease.

It is likely that many of these potential mechanisms interact to mediate the effects of radiation, and that the final effects of these processes are similar. For example, cell death of myocytes may lead to heart fibrosis, whilst microvascular damage in the pericardium may lead to pericardial fibrosis. One of the potential pathways through which radiation may mediate its effects is by the development of atherosclerosis (Figure 6.1). It may be that radiation is an additional risk factor for atherosclerosis or that it contributes to the other risk factors (see Chapter 2) or is an independent risk factor in its own right.

Finally, individuals may also be predisposed to developing radiation-induced or potentiated vascular disease, by having pre-existing DNA damage in diseased tissue or genetic predisposition due to inherited defects in DNA repair.

6.1 Direct and Indirect Effects of Radiation on the Cardiovascular System

The development of cardiovascular disease, in particular atherosclerosis, is a complex process that probably represents a balance between agents that cause cell damage and the body's ability to repair this damage. Anything that upsets this balance has the potential to initiate disease. While a full understanding of the process of atherosclerosis is not available and the effects of radiation are not well characterised, it

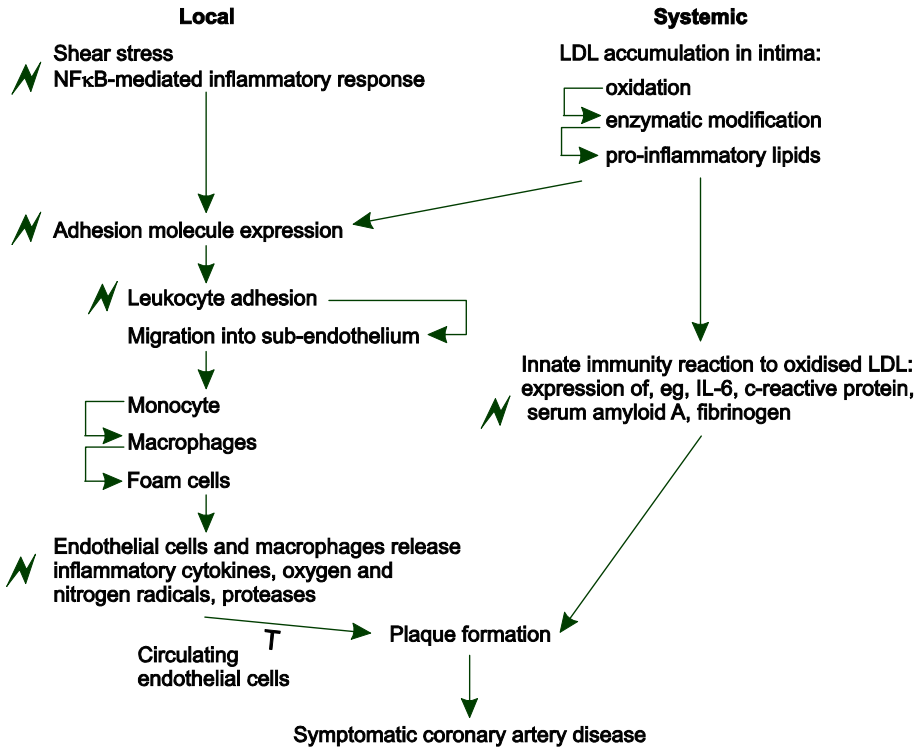


FIGURE 6.1 Schematic representation of the most important steps of pathogenesis coronary artery disease. Events that have also been observed after radiation exposure are indicated by flashes. (From Schultz-Hector and Trott, 2007)

is reasonable to suggest that the effects of radiation exposure on the circulatory system can be divided, broadly speaking, into those that are direct and those that are indirect, as follows.

a Direct effects For example, increased incidence of coronary artery disease seen when the coronary vessels are included in the radiation field for breast cancer treatment (Roychoudhuri et al, 2007).

b Indirect effects (also known as abscopal effects) For example, the release of inflammatory mediators into the circulatory system by radiation exposure to an organ distant to the cardiovascular system causing increased damage to endothelial cells and other critical cell populations.

c Indirect effects Reduction in protective mechanisms (eg endothelial progenitor cells) by radiation exposure and cell death in organs that are capable of releasing such cells into the circulatory system, upsetting the balance of damage and repair to endothelial cells and other critical cell populations.

Ultimately the individual mechanisms described below could influence all of these processes.

6.2 Potential Mechanisms of Radiation Changes at the Cellular, Subcellular and Molecular Levels

6.2.1 DNA damage

6.2.1.1 Pre-existing DNA damage in atherosclerosis

Adults exposed to radiation in occupational and medical settings frequently have diseased arteries; both circulating cells and the plaques themselves exhibit genetic damage that occurs as part of the pathogenesis of the disease. For example, circulating lymphocytes of patients with coronary artery disease have a higher micronucleus index (a marker of genetic instability) than healthy controls, which correlates with disease severity (Botto et al, 2001; Andreassi, 2003), and a significantly higher incidence and extent of a common mitochondrial DNA deletion (mtDNA4977) (Botto et al, 2005). Atherosclerotic plaques also manifest 'macro'-scale genomic damage. For example, cytogenetic analysis of primary cell cultures from human plaques identified multiple abnormalities, including loss of the Y chromosome and del(13q14), XXY karyotype, and trisomy of chromosomes 7, 10 and 18 (Casalone et al, 1991), whilst unstable carotid plaques demonstrated trisomy and tetrasomy of chromosome 7, and monosomy of chromosome 11 (Matturri et al, 1997, 2001). Although it was not clear from the studies above which cells had chromosomal defects, vascular smooth muscle cells (VSMCs) demonstrate microsatellite instability (mutations in microsatellite regions that may affect gene expression) (Hatzistamou et al, 1996; Spandidos et al, 1996; McCaffrey et al, 1997) and loss of heterozygosity (Hatzistamou et al, 1996), including in genes that regulate DNA repair (Flouris et al, 2000) and that regulate leukocyte adhesion, VSMC growth, differentiation and migration (Arvanitis et al, 2005).

This 'macro'-scale genomic damage is associated with biomarkers of carcinogen exposure, such as DNA adducts or modifications to specific bases in atherosclerotic plaques. 'Bulky' aromatic DNA adducts in VSMCs (most likely related to environmental exposure to genotoxic chemicals) are a predictor of atherosclerosis extent in humans even after adjustment for age, smoking, obesity, heart weight and genetic susceptibility markers (Binkova et al, 2002). DNA strand breaks, oxidised pyrimidines and altered purines are also significantly higher in patients with coronary artery disease than in controls (Botto et al, 2002), and human plaques show markers of oxidative damage, including DNA strand breaks, expression of 8-oxo-G (an oxidative modification of guanine residues in DNA) and activation of DNA repair enzymes (Martinet et al, 2002; Matthews et al, 2006). Strong nuclear and cytoplasmic immunoreactivity for 8-oxo-G is detected in plaque VSMCs, macrophages and endothelial cells, but not in VSMCs of adjacent normal media or normal arteries (Martinet et al, 2002). DNA damage is also a direct correlate of the extent of atherosclerosis in experimental animals. For example, cholesterol feeding of rabbits induces oxidative damage in plaques, manifested by 8-oxo-G staining (Martinet et al, 2001), DNA strand breaks and apoptosis.

These studies suggest that irradiation may exacerbate pre-existing genetic damage in atherosclerosis that has developed as part of the disease process. Indeed, the multiplicity of genetic defects is consistent with random DNA damage induced by oxidant stress or other agents (see below). DNA damage can also occur in the mitochondria, and mitochondrial DNA damage correlates with the extent of atherosclerosis in human specimens and aortas from apolipoprotein E-/- (ApoE-/-) mice (Ballinger et al, 2002). In contrast, the extent to which irradiation of already damaged cells in atherosclerosis exacerbates DNA damage is not known.

6.2.1.2 Genetic predisposition resulting from defects in DNA repair genes

DNA damage induces a cascade of activated proteins that act as sensors and effectors of the damage response, to stall the cell cycle allowing repair to occur, to promote repair, or to induce apoptosis if damage is severe (reviewed by Mahmoudi et al, 2006). The sensor proteins for the key radiation-induced lesion, the DNA double strand break (DSB), include Nijmegen Breakage Syndrome-1 (NBS-1), a ubiquitously expressed 754 amino acid protein and key regulator of the MRE11/RAD-50/NBS-1 (MRN) complex (Carney et al, 1998; Varon et al, 1998). MRN promotes early processing of DSBs via DNA binding and nuclease activities, functions as a DSB sensor and also recruits ataxia-telangiectasia-mutated protein (ATM) to DSBs, followed by ATM activation (Paull and Gellert, 1999; Falck et al, 2005; Lee and Paull, 2005). ATM is normally present in cells as inactive dimers, but DSB exposure induces autophosphorylation at ser^{1981}, dimer dissociation and kinase activation. ATM has multiple downstream substrates that mediate cell senescence, growth arrest and apoptosis, including histone 2AX (H2AX) and specific checkpoint kinases, such as Chk2 and Chk1, which in turn phosphorylate p53 (Chehab et al, 2000; Shieh et al, 2000). ATM accumulates at DSBs, as marked by ser^{139} phosphorylation of H2AX flanking the site of DNA damage (Shiloh, 2003). Phosphorylated histone H2AX (γ-H2AX) facilitates the local assembly of checkpoint and DNA repair factors, and is a robust marker of DSBs (Stucki and Jackson, 2006). Growth arrest following DNA damage is partly mediated by p53 and its target genes, such as p21, although p21 can also induce growth arrest following DNA damage independently of p53 (Macleod et al, 1995).

Human atherosclerotic plaques show increased expression of many of these DNA repair markers, including ser^{1981}ATM, γ-H2AX, redox factor/AP endonuclease, poly(ADP-ribose) polymerase 1 (PARP-1), and DNA-dependent protein kinase (Martinet et al, 2002; Mahmoudi et al, 2008), with levels increasing with disease severity. Many of these markers increase with fat feeding in animal models of atherosclerosis, and are reduced by normalisation of the diet or treatment with drugs such as the HMGCoA reductase inhibitors (statins) (Martinet et al, 2001; Mahmoudi et al, 2008). Although little is known about the ability of normal or diseased vascular cells to repair oxidative DNA damage, plaque VSMCs show persistent DNA strand breaks and activation of DNA repair pathways *in vitro*, suggesting either ongoing damage or ineffective repair (Mahmoudi et al, 2008). In contrast, normal VSMCs show a robust and rapid repair of oxidative damage (Mahmoudi et al, 2008), the kinetics of which are influenced by expression levels of the key repair proteins.

There is limited evidence that hereditary defects in genes that regulate responses to DNA damage predispose to premature atherosclerosis. Premature atherosclerosis is a feature of the Werner syndrome, a disease characterised by predisposition to cancer and early onset of features seen in normal ageing, including osteoporosis, ocular cataracts, greying and loss of hair, diabetes mellitus and atherosclerosis. Werner protein guards the genetic stability of cells, playing an integral role in base excision repair and at telomere ends. In addition, most patients with Hutchinson Gilford Progeria Syndrome (HGPS), an accelerated ageing syndrome, die of atherosclerosis. VSMC depletion is a major feature in progeria and normal ageing, and is likely to represent replicative senescence, telomere shortening and decreased capacity for repair, as HGPS VSMCs are more susceptible to haemodynamic and ischaemic stress (Stehbens et al, 1999) (see below). In other studies, individuals heterozygous for mutations in ATM were reported to be twice as likely to die from myocardial infarction than normal controls (Swift and Chase, 1983; Swift et al, 1987; Su and Swift, 2000). Although subsequent work primarily focused on cancer

incidence found no evidence for an increase in mortality from circulatory disease (Thompson et al, 2005), mice heterozygous for ATM showed accelerated atherosclerosis, with multiple features of the metabolic syndrome (Schneider et al, 2006).

In addition to these relatively rare syndromes, there are reports from one group of a possible genetic susceptibility to radiation-induced atherosclerosis (Hannan et al, 1994). Fibroblasts from five patients with severe atherosclerosis exhibited a clonogenic sensitivity to chronic (low dose rate) irradiation that was intermediate between that of healthy subjects and that of patients with the known radiosensitive syndrome ataxia telangiectasia (AT). In a subsequent study with 19 atherosclerosis patients, 3 normals, 2 AT individuals, and 3 heterozygotes (ATH), there was a considerable interstrain difference in the radiosensitivity of fibroblasts from patients, with their mean D_{10}* values varying between 2.3 and 6.2 Gy, whereas the mean D_{10} values for the cells from the AT homozygote, AT heterozygotes and healthy subjects were 2.0, 3.8 and 9.0 Gy, respectively (Nasrin et al, 1997). A majority of the cell strains from atherosclerosis patients exhibited varying degrees of radioresistant DNA synthesis (RDS), with roughly 33% showing an AT-like and the rest an ATH-like response. Although these studies suggest that patients with coronary artery disease have reduced radiosensitivity, these observations have not been confirmed or refuted by other groups, and cellular hyposensitivity to radiation is rare among the general population – the proportion of individuals with a cellular radiosensitivity more than two standard deviations from the mean is around 4% (Arlett et al, 2008).

6.2.2 Mutation and cell transformation

The vascular smooth muscle cells (VSMCs) in the fibrous cap of the atherosclerotic plaques are monoclonal (Benditt and Benditt, 1973; Pearson et al, 1987; Murry et al, 1997; Chung et al, 1998), as are tumours, meaning that they preferentially express the same cell lineage marker, rather than the random expression of such markers that would be expected from a multiclonal origin. Clonality argues that plaque VSMCs must have undergone multiple rounds of division, and telomere loss studies argue that this is between seven and thirteen cumulative population doublings (Matthews et al, 2006). These observations raised the possibility that spontaneous plaques (or at least their fibrous caps) arise from a subset of VSMCs that have a proliferative/survival advantage over the rest of the medial cells. This could either be a dominant transforming mutation in a single cell akin to tumourigenesis, or a more subtle mutation that alters a signalling pathway for cell division/growth arrest.

In support of this concept, there have been reports of mutations, transforming activity and differential gene expression in cells from plaques compared with normal vessels. McCaffrey et al (1997) found mutations in a microsatellite sequence in the Type II TGF-β1 receptor in plaque-derived cells, leading to a premature truncation that would cause loss of normal growth inhibition by TGF-β1. Similarly, Matturri et al (1997) showed trisomy 7 in plaques and correlated this observation with overexpression of the PDGF-A gene and subsequent increased cell proliferation. Studies on DNA isolated from human plaques have also led to comparisons with carcinogenesis. Penn and colleagues found transforming activity of cells derived

* D_{10} is the radiation dose resulting in survival of 10% of cells.

from human plaques in an NIH 3T3 assay and *in vivo* in nude mice (Penn et al, 1986), and from carcinogen-induced plaques from cockerels (Penn et al, 1991). These workers later demonstrated that cells cultured from two human plaques overexpress the proto-oncogene c-myc compared with normal medial cells, and plaque VSMC DNA demonstrated transforming activity, although c-myc did not appear to be the transforming gene (Parkes et al, 1991).This transformation work has not been directly replicated and the nature of the apparent activity remains unknown. In another study, Speir et al (1994) found that 38% of specimens from angioplasty restenosis sites expressed high levels of p53, and this correlated with expression of cytomegalovirus products, one of which can inactivate p53.

In contrast, despite their fundamental relevance, these studies have not been confirmed, and in many cases identical studies have found opposite results. For example, TGF-β1 microsatellite stability was found to be normal in atherosclerosis (Bobik et al, 1999; Clark et al, 2001), and although plaque VSMCs have a very different phenotype and gene expression compared with cells from the normal vessel wall (Schwartz et al, 1995; Mulvihill et al, 2004), and carry damaged DNA (see above), there is very little consistent evidence that plaques carry mutations with pro-proliferative or anti-apoptotic phenotypes similar to those seen in carcinogenesis. For example, DNA isolated from human atherosclerotic plaques does not show mutations in the common oncogenes (eg Ras and c-myc) (Parkes et al, 1991; Kiaris et al, 1996). In addition, p53 expression and activity is either normal or increased in atherosclerosis (Ihling et al, 1997, 1998; Kavurma et al, 2007) and plaque VSMCs demonstrate normal p53 structure with no evidence of mutation (Iacopetta et al, 1995), compared to cancer where over 50% of cancers show a mutation or loss of p53. Furthermore, although plaques can be produced in chickens with the oncogenic lymphotropic herpesvirus responsible for Marek's disease, this appeared to be due at least in part to an alteration of cholesterol metabolism rather than transforming mutations in VSMCs (Fabricant et al, 1978; Hajjar et al, 1986).

In addition, clonality itself is not synonymous with transformation of a single cell, and subsequent studies have shown that large patches in the normal vessel media are monoclonal (Chung et al, 1998). Thus, clonality may be explained by the presence of developmental clones in the normal vessel wall, rather than a mutation conferring proliferative advantage. As described below and in contrast to tumours, plaque VSMCs show poor proliferation, enhanced apoptosis and early senescence (Schwartz and Murry, 1998; Kavurma et al, 2007). These features would not confer a proliferative or survival advantage. Furthermore, as described below, plaque VSMC proliferation is now seen to be beneficial in atherosclerosis, so that the pathological consequences of a mutation promoting VSMC proliferation are unclear.

Rather than processes akin to carcinogenesis, mutations in atherosclerosis are more likely to be a secondary event, or an epigenetic phenomenon. Lipid oxidation and reactive oxidant species are potent stimuli inducing DNA damage, as are numerous risk factors for coronary artery disease, including smoking and diabetes. For example, smoking can cause oxidative DNA damage, inhibit DNA repair and induce the production of advanced glycation endproducts, which themselves cause DNA mutation (reviewed by Basta et al, 2004). Similarly, advanced glycation endproducts have been implicated in the oxidation of low density lipoprotein (Bucala et al, 1993) and elevated levels of 8-oxo-G.

In summary, VSMCs in advanced human plaques have neither the functional properties nor the DNA mutations seen in carcinogenesis. The presence of clonality does not infer that a process akin to carcinogenesis results in spontaneous human atherosclerosis. Whilst irradiation may promote further DNA damage, at present we have no evidence that any potentiation of atherosclerosis is due to cellular changes that are known to be involved in carcinogenesis. If there are persisting changes in DNA that have a causal role in plaque formation there is no reason to believe that they would be detectable *in vitro* by cellular transformation systems designed to detect carcinogenic mutations.

6.2.3 Cell proliferation and cell senescence

In the 1970s, pioneering work using animal models of vascular injury (not specifically radiation injury) resulted in the 'response to injury' model of atherosclerosis, in which plaques develop following stimuli that physically or functionally injure the vessel wall (see Ross and Glomset, 1973, and subsequent reviews). A fundamental principle underlying the 'response to injury' hypothesis is that VSMC proliferation promotes the development of the plaque. If DNA damage gives a cell a selective proliferation advantage (see above), such damage might be predicted to promote atherosclerosis. In contrast, whilst VSMC proliferation underlies the response of normal arteries to a variety of injurious stimuli, the biology of human atherosclerosis is very different. In particular, rather than a selective growth advantage, human plaque VSMCs show numerous markers of cell senescence (Ross et al, 1984; Mosse et al, 1985; Bennett et al, 1995; Bauriedel et al, 1999), including impaired proliferation (Ross et al, 1984; Mosse et al, 1985; Bennett et al, 1995, 1998; Bonin et al, 1999), early culture senescence (Bennett et al, 1995, 1998; Bonin et al, 1999), and both *in vitro* and *in vivo* markers of senescence, including morphological features (Goldstein, 1990), increased expression of replication checkpoint genes (p16 and p21) (Ihling et al, 1997; Tanner et al, 1998; O'Sullivan et al, 2003; Matthews et al, 2006) and senescence-associated β-galactosidase activity. Plaque VSMCs in culture and *in vivo* also show extensive telomere loss, barely detectable telomerase activity and telomere lengths predicted to activate senescence (Matthews et al, 2006).

Whilst these findings effectively exclude a causal role for a dominantly transforming process akin to carcinogenesis promoting atherosclerosis, cell senescence may itself be a major driver for atherosclerosis development. In recent years the role of cell proliferation in atherogenesis has been re-evaluated. Cell proliferation in advanced disease is now seen as protective, to repair the subclinically ruptured plaque. Indeed, the presence of VSMCs retards the development of atherosclerosis even in early lesions (Clarke et al, 2008). Thus, rather than giving a growth advantage, irradiation could directly accelerate the progression of established atherosclerosis and its clinical consequences by inducing cell senescence. Whilst it is unclear whether premature cell senescence in earlier lesions also promotes atherogenesis, extensive data from human conditions associated with premature ageing, such as Hutchinson Gilford Progeria (HGP) (Varga et al, 2006) and Werner syndrome (Brown, 1992) – both of which show extensive DNA damage in vessel cells – are associated with both premature cellular senescence and accelerated atherosclerosis. Indeed, HGP mutations predominantly affect VSMCs in the vessel wall, causing medial atrophy and premature atherosclerosis (McClintock et al, 2006). Thus, DNA damage and cell senescence are causally associated with atherosclerosis.

Premature senescence of other cells, particularly endothelial cells (ECs), has also been observed in atherosclerosis (Vasile et al, 2001; Minamino et al, 2003), and there is extensive evidence that EC senescence contributes to atherogenesis [reviewed by Minamino et al (2002), d'Alessio (2004), Minamino et al (2004), Brandes et al (2005), Erusalimsky and Kurz (2005), and Freedman (2005)]. In particular, EC turnover is most marked at sites where atherosclerosis develops later. Similar to VSMCs, an inability to proliferate and repair de-endothelialised sites can promote atherosclerosis, via, for example, loss of a functional endothelial cell barrier to inflammatory cell invasion.

6.2.4 Cell death

Cell death via apoptosis is frequently observed both *in vitro* and *in vivo* after irradiation, and is activated when DNA damage is too severe to be repaired. Irradiation of the heart may induce the death of cells of the heart itself (cardiomyocytes, cardiac fibroblasts and conducting tissue) and the death of cells of contained vessels (both capillaries and epicardial vessels). In general, the cells undergoing radiation-induced apoptosis are those closest to the source (that is, those receiving the highest dose) or those that are the most actively proliferating, particularly endothelial cells. Whilst isolated VSMCs have been reported to be more sensitive to radiation than isolated ECs (Kotzerke et al, 2000), atherosclerosis itself increases the sensitivity of both ECs and VSMCs to apoptosis, via alterations in the extracellular milieu – in particular, the presence of inflammatory cells and the balance of pro- and anti-apoptotic cytokines. In addition, plaque VSMCs show increased intrinsic sensitivity to apoptosis compared with normal VSMCs, both *in vitro* and *in vivo* (Bennett et al, 1995). Increased sensitivity occurs via overexpression/activation of p53 (Bennett et al, 1997), with subsequent reduction in cell protection signalling (Patel et al, 2001; Kavurma et al, 2007).

EC apoptosis appears to be central to radiation-induced vascular injury (see above) and radiation is a potent inducer of EC apoptosis *in vitro*, which can be protected by the presence of serum or growth factors such as basic fibroblast growth factor (bFGF) (Fuks et al, 1994; Langley et al, 1995). Basic fibroblast growth factor also protects vessels from radiation-induced stenosis after chest irradiation, thus adding weight to the argument that EC apoptosis also underlies vessel injury after irradiation (Fuks et al, 1994). Indeed, the direct consequences of vascular cell apoptosis have recently been elucidated. In general, acute EC apoptosis can induce thrombosis (Durand et al, 2004), whilst chronic EC apoptosis can promote atherosclerosis. Similarly, VSMC apoptosis accelerates both atherogenesis and the progression of established plaques (Clarke et al, 2008), and promotes multiple features of vulnerable plaques (Clarke et al, 2006). Thus, irradiation-induced VSMC apoptosis in diseased arteries might be predicted to significantly increase atherosclerosis and plaque rupture.

The third cell type that readily undergoes apoptosis in atherosclerosis is the macrophage, and macrophages comprise the most frequently identifiable cell type undergoing apoptosis in advanced lesions (Lutgens et al, 1999). However, macrophage apoptosis has different effects on atherosclerosis at different points in the disease process. Macrophage apoptosis in atherogenesis reduces lesion formation (Stoneman et al, 2007), whereas apoptosis in established plaques may promote necrotic core formation and inflammation, contributing to plaque instability (reviewed by Tabas, 2005). The effects of radiation-induced macrophage apoptosis in atherosclerosis are currently unknown.

6.2.5 Mitochondrial dysfunction

Mitochondrial DNA (MtDNA) is very vulnerable to DNA damage in all cell types including vascular cells (Ballinger et al, 2000), in part because it lacks protective histones, and its close proximity to the inner mitochondrial membrane. MtDNA damage also persists longer than nuclear DNA damage (Clayton et al, 1974; Yakes and Van Houten, 1997; Croteau et al, 1999) due to the lack of specific nuclear DNA repair pathways, particularly nucleotide excision repair. MtDNA damage can itself lead to increased reactive oxygen species (ROS) production through inefficient oxidative phosphorylation (Pitkanen and Robinson, 1996; Chomyn and Attardi, 2003), and ROS can damage MtDNA, resulting in a positive feedback loop. MtDNA damage ultimately leads to both cellular senescence and apoptosis, even in the absence of increased ROS production (Trifunovic et al, 2005), and is a major mechanism invoked in the free-radical theory of ageing (Trifunovic et al, 2004; Kujoth et al, 2005). Damage to the mitochondrial genome is a frequent observation in human atherosclerosis in circulating cells and cells of the vessel wall, particularly a specific 4977-bp deletion, known as the common deletion – Delta-MtDNA(4977) – associated with mitochondrial dysfunction. Mitochondrial genome damage correlates with the extent of atherosclerosis in humans and Apolipoprotein E-/- mice and precedes atherogenesis in young ApoE-/- mice, suggesting a causal role. Indeed, ApoE-/- mice deficient in manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme, show early increases in MtDNA damage and accelerated atherogenesis (Ballinger et al, 2002), suggesting that increased MtDNA damage and ROS production is an early event in atherosclerosis. Although there are limited studies on the role of irradiation and MtDNA damage in atherosclerosis, irradiation can produce the common MtDNA mutation (Prithivirajsingh et al, 2004).

6.2.6 Thrombosis

Radiation-induced vascular injury is known to activate several prothrombotic events in normal tissues leading to fibrin deposition and thrombus formation in microvessels (Fajardo, 1999, 2005). Prothrombotic effects of radiation have been shown in endothelial cells *in vitro* and in *in vivo* models. Radiation was shown to cause a reduction in the anti-coagulant protein thrombomodulin in endothelial cells in culture (Zhou et al, 1992) and in the irradiated rat intestine (Richter et al, 1997; Wang et al, 2002; Van der Meeren, 2003). Radiation also elevates production of procoagulant tissue factor (Verheij et al, 1995) and expression and release of the haemostatic protein, von Willebrand factor, in cultured endothelial cells (Sporn et al, 1984; Jahroudi et al, 1996; Verheij et al, 1997). In addition, von Willebrand factor is known to be elevated in irradiated kidney and lung *in vivo* (van Kleef et al, 1998). Prothrombotic changes in endothelial cells triggered by radiation also include decreased production of prostacyclin and ADPase activity (Ts'ao et al, 1983a; Verheij et al, 1994; te Poele et al, 2001). Radiation also causes a decrease in fibrinolytic activity both *in vivo* and *in vitro* (Ts'ao et al, 1983b). Evidence for thrombosis specifically in the heart has been demonstrated by early studies of Fajardo and colleagues that detected microthrombi and platelet aggregates in the microvasculature of irradiated rabbits (Brown et al, 1973; Fajardo and Stewart, 1973). More recent studies by Boerma et al (2004) showed deposition or release of von Willebrand factor in rat heart capillaries after one month of irradiation, indicative of procoagulant activity and endothelial cell damage. Increased levels of von Willebrand factor were still present three months after irradiation within the matrix of fibrotic areas, suggesting a role in fibrosis.

Irradiation can induce subclinical arterial damage sufficient to promote arterial thrombosis, even without the development of atherosclerosis (Call et al, 1990). This may be due to direct damage to ECs, with a reduction in their ability to produce prostacyclin (PGI2). For example, irradiation induces dose- and time-related damage to enzymes of the arachidonic acid cascade, associated with oxidant stress and production of free radicals (Eldor et al, 1989). Taken together therefore these experimental and clinical data are suggestive of a crucial role for coagulation in the vascular damaging effects of radiation.

6.2.7 Inflammation

Although inflammation is suspected to play a crucial role in all stages of atherosclerosis (Hansson, 2005), and is known to occur following radiation exposure (Hayashi et al, 2003, 2005), there are to date no direct studies that link radiation exposure to an increased risk of cardiovascular disease via the intermediary of inflammation. However, it can be assumed that inflammation plays an important role in the causal mechanistic pathways that link radiation exposure to cardiovascular disease.

6.2.7.1 Evidence for the role of radiation-induced inflammation

Evidence for inflammation causing cardiovascular disease comes from observation of elevated systemic markers of inflammation, such as C-reactive protein (CRP) (Ridker et al, 2000a; Tzoulaki et al, 2005), interleukin 6 (IL-6) (Ridker et al, 2000b; Tzoulaki et al, 2005), vascular cell adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecules (eg E-selectin) in patients experiencing cardiac events compared with control subjects. These observations are further supported by many large-scale prospective studies that have now shown that inflammatory biomarkers, such as high sensitivity C-reactive protein (hsCRP), are independent predictors of future cardiovascular events (Packard and Libby, 2008). Indeed, studies have shown that inflammation (as reflected by elevated hsCRP) precedes cardiovascular events, and is not a result of other risk factors, stressing that atherosclerosis is, in part, an inflammatory disease (Ridker et al, 1997, 1998; Ross, 1999; Hansson, 2005; Libby and Ridker, 2006). Although these markers are associated with an increased risk of cardiovascular disease, no direct causal link has been established.

There is much evidence from animal studies and observations of radiotherapy patients that radiation exposure causes damage to the heart (see Chapter 5). In experimental studies, inflammation has been shown to be activated via the up-regulation of E-selectin secondary to radiation exposure (Hallahan et al, 1995). This adhesion molecule is released by the endothelial cells and causes increased leukocyte adhesion – an essential step in the inflammatory process. The process can be inhibited by knockout of the E-selectin binding site. This effect has been observed at doses as low as 0.5 Gy. Irradiation of endothelial cells at 1–5 Gy promotes activation of transcription factor activator protein 1 and NF-κB, followed by increased expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and platelet-endothelial cell adhesion molecule (PECAM), and overproduction of monocyte chemo-attractant protein 1, IL-6, IL-8, FGF-2, and IL-1β. Up-regulation of some cytokines (IL-6 and IL-8) has been observed after endothelial cell irradiation in a time- and dose-related fashion (Van der Meeren et al, 1999). Irradiation also increases mRNA levels of TGF-β1, PDGF-A and PDGF-B about 1.2-fold, and promotes increased adhesion of leukocytes and platelets (Wondergem et al, 2004; Gaugler et al, 2005). Irradiation of VSMCs

can also promote ICAM-1 expression (Voisard et al, 2007). These studies all indicate that irradiation may be directly pro-inflammatory to cells of the vessel wall, promoting leukocyte adhesion and migration, and could underline the transient nature of the pro-atherogenic effect of acute irradiation (Wong et al, 1999). Similarly, animal studies at the 1–5 Gy level reveal increased expression of adhesion molecules in the cardiovascular endothelium and other vasculature (Hallahan and Virudachalam, 1997a; Schultz-Hector and Trott, 2007) – ICAM-1 in the microvasculature and E-selectin in the endothelium of the large blood vessels (Hallahan and Virudachalam, 1997b). Interestingly, radiation can lead to an increase in endothelial cell permeability, and increased accumulation of lipids in the presence of hypercholesterolaemia (Tribble et al, 1999). Also, ionising radiation shifts the balance from ALK1 to ALK5 signalling and activates the Notch signalling pathway in endothelial cells. This combination of anti-angiogenic signals contributes to reduced cell migration after irradiation (Scharpfenecker et al, 2009). These lines of evidence raise the possibility that radiation at these doses could not only play a role in the initiation of atherosclerosis, but also play a role in the acceleration of plaque formation in those where disease is already present (see Figure 5.3). In humans, elevated levels of both pro-inflammatory agents such as CRP, IL-6, tumour necrosis factor (TNF) and interferon (INF), and anti-inflammatory cytokine IL-10, have been seen in the Japanese atomic bomb survivors (Hayashi et al, 2003, 2005).

Irradiation of inflammatory cells may also have pro-atherogenic effects. For example, irradiation of macrophage colony-stimulating factor-activated human peripheral blood monocytes results in enhanced expression of CD36 scavenger receptors and cholesterol accumulation with resultant foam cell formation in the presence of oxidised low density lipoprotein. Furthermore, when cultured on collagen gels, human macrophages formed large foam cell aggregates in response to irradiation. These effects were dependent upon c-Jun N-terminal kinase activation and CD36 (Katayama et al, 2008).

In contrast to the up-regulation seen at high doses, acute doses in the range of 0.1–1 Gy may result in the down-regulation of leukocyte adhesion to the endothelium and hence have an anti-inflammatory effect (Kern et al, 2000; Hosoi et al, 2001; Roedel et al, 2002). These observation are also seen in animal studies (Hildebrant et al, 1998), and go some way to supporting the role of low to medium dose radiation for the treatment of inflammatory conditions in humans (Trott, 1994). However, reduction in macrophage nitric oxide (NO) production has also been observed at low doses. Nitric oxide appears to be an endothelial atheroprotective molecule, and has been observed to be reduced, with concomitant increase in VCAM-1 expression, at site of arterial bifurcation, where atherosclerosis is common (Jongstra-Bilen et al, 2006).

6.2.7.2 Evidence for the role of inflammation in cardiovascular disease

Over the last decade, our understanding of the pathophysiology of the development of atherosclerosis has been underpinned by the 'cholesterol hypothesis' (Tabas, 2002). Hypercholesterolaemia is a risk factor for human atherosclerosis, and animals with disruption of lipid uptake resulting in hypercholesterolaemia also develop atherosclerosis. Cholesterol and cholesterol esters are found in the vessel wall in very early stages of atherosclerosis, and drugs that lower cholesterol reduce cardiovascular clinical events. Despite the important role for cholesterol, many people who experience symptomatic atherosclerosis (stroke or myocardial infarction) do not have elevated blood cholesterol, suggesting that cholesterol is only one of the components contributing to atherosclerosis. In particular, inflammation within the arterial wall is an

important part of the process that initiates atherosclerosis, its progression and complications (Glass and Witztum, 2001). Importantly, inflammation can be seen as a unifying mechanism for the action of many of the 'risk factors' for cardiovascular disease – smoking, diabetes and hypertension (Libby and Aikawa, 2002) (Figure 6.2).

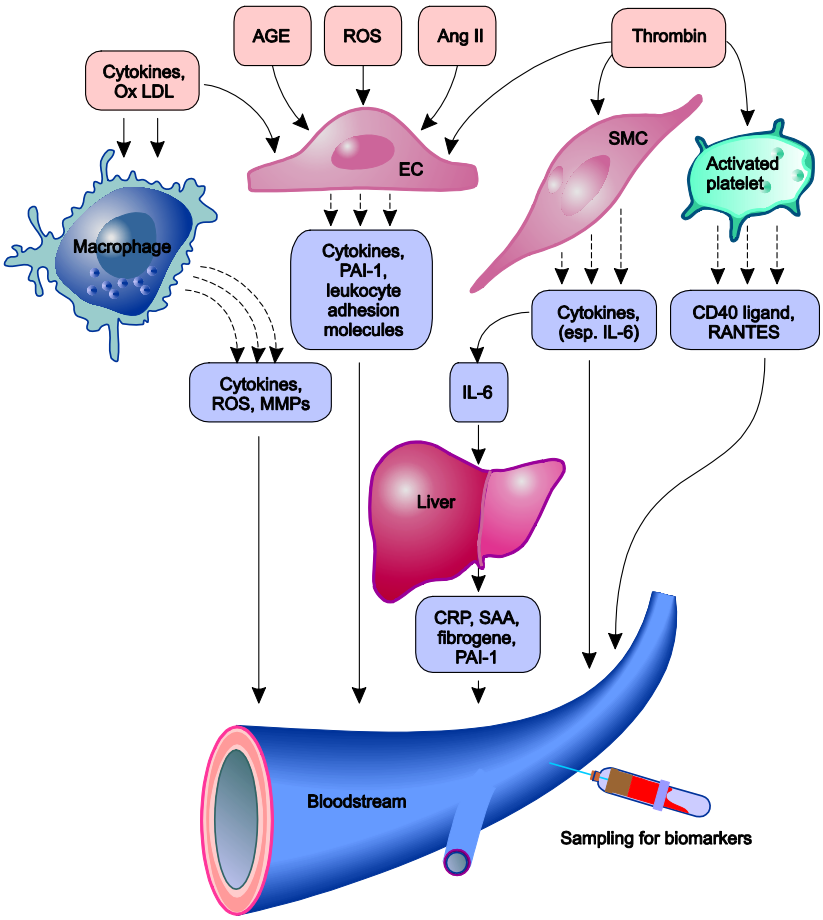


FIGURE 6.2 Inflammation links classic risk factors to altered cellular behaviour within the arterial wall and secretion of inflammatory markers in the circulation

Primary pro-inflammatory risk factors elicit the expression of primary pro-inflammatory cytokines that can be released directly into the blood. Cytokines orchestrate the production of adhesion molecules, matrix metalloproteinases, and reactive oxygen species that may also be released from lesions. In parallel, these primary cytokines induce the expression of the messenger cytokine IL-6, particularly in smooth muscle cells. IL-6 then travels to the liver, where it elicits the acute-phase response, resulting in the release of C-reactive protein, fibrinogen, and plasminogen activator inhibitor-1. All these inflammatory markers and mediators, released at different stages in the pathobiology of atherothrombosis, can enter the circulation, where they can be easily measured in a peripheral vein. AGE, advanced glycation endproducts; Ang II, angiotensin II; OxLDL, oxidised low density lipoprotein; RANTES, regulated on activation, normal T-cell expressed and secreted; ROS, reactive oxygen species; SAA, serum amyloid A. (From Hansson, 2005)

Many of the factors that have been described as being activated by radiation exposure (VCAM-1, E-selection and nitric oxide) are believed to be involved in the initiation of atherosclerosis, precipitating cellular infiltration by macrophages (Rajavashisth et al, 1990; Clinton et al, 1992) and T-cells (Stemme et al, 1995), leading to the formation of fatty streaks. Progression to atheromatous lesions is also driven by pro-inflammatory factors – eg adhesion molecules (VCAM-1), chemokines (IL-8), cytokines (IL-6) and matrix metalloproteinases (MMP-1/-9/-13) – which can contribute to smooth muscle cell proliferation/migration and the formation of fibrous plaques. At the same time, inflammatory factors can stimulate neo-vascularisation from the arterial vasa vasorum, enabling further recruitment of inflammatory agents and cells to the lesions. Finally, plaque rupture itself now appears to be secondary to inflammation (Libby, 2001). Those plaques that are prone to rupture have a paucity of smooth muscle cells, and hence a thin plaque 'fibrous cap', and an abundance of macrophages. It is rupture of these lesions that can lead to fatal arterial thrombosis and occlusion. Inflammation appears to play a key role in the thinning of the fibrous cap by interfering with collagen maintenance (increased destruction) and formation (reduced production) (Amento et al, 1991), and may also directly promote VSMC death (Boyle et al, 2001). From these mechanisms, it can be seen that radiation-induced inflammation may act not only to promote atherogenesis, but also to accelerate the progression of existing atheromatous lesions. It should be noted, however, that acceleration of plaque formation in the irradiated ApoE-/- mouse model has also been associated with increased macrophage and granulocyte numbers in plaques providing a local inflammatory environment (Stewart et al, 2006; Hoving et al, 2008).

6.2.7.3 Distant release of inflammatory factors

As explained above, inflammation has a direct effect on the arterial wall. Local radiation-induced inflammation may therefore play a direct role in potentiating this inflammatory response at the site of radiation exposure. However, there is increasing evidence that inflammatory factors released into the circulatory system by more distant inflammatory processes can lead to observed changes in endothelial function (Giannotti and Landmesser, 2007). Furthermore, as endothelial function or 'health' is probably a balance between endothelial damage and endothelial repair (possibly via progenitor cells), effects of radiation that release inflammatory agents into the circulation (eg secondary to any radiation-induced cell death) or reduce the number of circulating repair cells (eg radiation to the bone marrow) may lead to an increase in endothelial damage and hence potentiate the initiation or progress of atherosclerosis.

Over the last two decades, several surrogate markers/observations for atherosclerosis have been identified. One such assessment is by using brachial artery ultrasound to assess flow-mediated dilatation (FMD) (Al-Qais et al, 2008). Importantly, loss of endothelial-dependent vasodilatation is independently related to future adverse cardiovascular risk (Celermajer et al, 1992). FMD interrogates nitric-oxide-dependent endothelial function. As such, a reduction in FMD is regarded as showing a reduction in the protective influence of NO, and hence poor underlying endothelial 'health'. Assessment of FMD is used in the diagnosis of a variety of inflammatory states, including infection (Charakida et al, 2005a,b; Simmonds et al, 2008) and poor dental hygiene (Tonetti et al, 2007), providing a useful measure of reduced endothelial function associated with a high level of inflammatory markers. Other markers of vascular health, such as arterial stiffness (Jarvisalo et al, 2004) and carotid intima-media thickness (C-IMT) (Ellins et al, 2008), have also been associated with inflammatory response. Increases in arterial stiffness,

correlated with inflammatory mediators, have been observed after psychological stress (Baldassarre et al, 2008). Whilst increases in C-IMT have been associated with CRP, TNFα, IL-6 and a whole range of other inflammatory markers (Ellins et al, 2008).

The assessment of these surrogate markers may potentially act as a method to assess the link between inflammation and radiation exposure in future research studies.

6.2.7.4 Clinical significance of inflammation

Although a link between radiation-induced inflammation and the role of inflammation as a risk factor for cardiovascular disease is very suggestive, no studies have demonstrated a direct link between radiation-induced inflammation and cardiovascular disease. There is currently only one study that suggests a link between radiotherapy and an increased cardiovascular risk profile (Wethal et al, 2007). In this study patients were grouped as to whether they had surgery alone, radiotherapy alone, or chemotherapy with or without surgery for the treatment of testicular carcinoma. Serum inflammatory markers (CRP) and atherogenic lipoproteins were measured five to twenty years after treatment. The study showed that there was a significantly higher incidence of cardiovascular risk factors in both the radiotherapy and chemotherapy groups, with radiotherapy associated with elevated serum markers of chronic inflammation and endothelial dysfunction, and chemotherapy associated with the development of atherogenic lipid changes and the metabolic syndrome. Again, these data are suggestive of an inflammatory link between radiation exposure and cardiovascular disease.

6.2.8 Endothelial repair

The health of the vascular endothelium is determined by a balance between damage and repair (Giannotti and Landmesser, 2007) (Figure 6.3). Bone marrow derived endothelial progenitor cells (EPCs) may play an important role in this repair mechanism and re-endothelialisation following vascular injury, although the evidence that the bone marrow contributes to the endothelium over atherosclerotic plaques is controversial (Hagensen et al, 2010). In humans, circulating EPC numbers relate to physiological assessment of endothelial function (FMD) and cardiovascular outcome in a range of diseases (Murphy et al, 2007; Oliveras et al, 2008; Vaughn et al, 2008). Hence radiation damage to EPCs could upset the balance between inflammatory damage and endothelial repair.

A further mechanism that could upset this balance of vascular damage/repair is cell senescence and other forms of cell death (see also Sections 6.2.5 and 6.2.6 above). Recent evidence suggests that infection, inflammation and oxidative stress can lead to accelerated senescence. In established atherosclerosis, a broad range of cells have been reported to show senescence phenotype, including lymphocytes, macrophages, smooth muscle cells, EPCs (Thijssen et al, 2009) and endothelial cells, and this has been correlated with endothelial dysfunction and reduced nitric oxide bioavailability (Hill et al, 2003). Cell senescence is associated with radiation exposure (Verheij, 2008), and though more often thought of as a mechanism in the pathway of cancer cell generation (Yang, 2008), could have a role in reduction of bone marrow EPC production and hence swing the balance of endothelial damage/repair towards damage and the development of atherosclerosis.

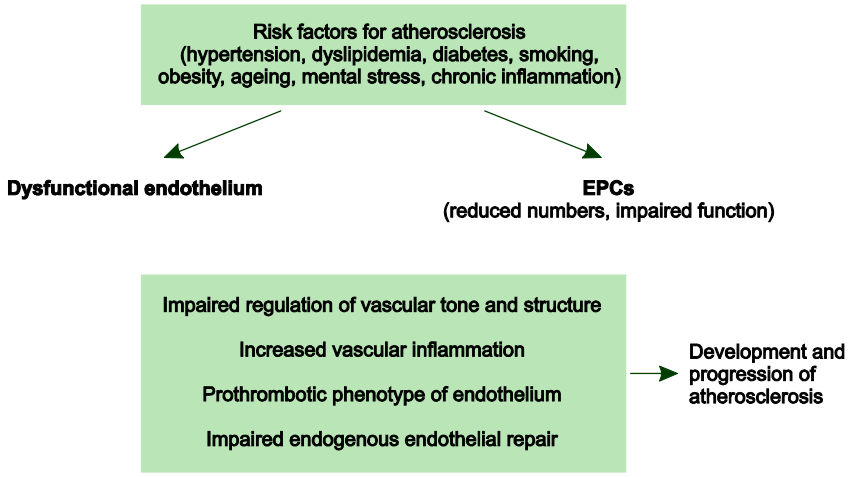


FIGURE 6.3 Cardiovascular risk factors and the inflammatory cascade act on the healthy endothelium to promote a pro-inflammatory and prothrombotic phenotype of the endothelium and impair the mobilisation and function of endothelial progenitor cells (EPCs), mediated in part by a reduced endothelial cell and EPC nitric oxide (NO) availability due to increased vascular and systemic oxidant stress. The altered endothelium leads to an impaired endothelium-dependent vasodilation, and a pro-inflammatory and pro-thrombotic phenotype of the endothelium, promoting the development of atherosclerosis. Moreover, impaired EPC function alters the endogenous endothelial repair capacity. (From Giannotti and Landmesser, 2007)

6.2.9 Fibrosis and fibrotic signalling pathways

Fibrosis is a very common late manifestation of radiation injury characterised by progressive depletion of parenchyma cells, which are replaced by fibrous matrix deposited by recruited myofibroblasts, fibroblasts or smooth muscle cells (O'Sullivan and Levin, 2003). Excessive deposition of extracellular matrix proteins and particularly collagens leads to scarring and overgrowth of tissues and impairment of the function of organs including the heart and the circulation (Fajardo and Stewart, 1973; Rodemann and Bamberg, 1995; Fajardo, 2005). Fibrosis is a common feature of radiation damage in vascular beds. Telangiectasia, is characterised by dilated and fragile capillaries prone to bleeding and rupture and is attributed at least in part to fibrotic changes in the extracellular matrix. In arterioles and small-sized arteries, subendothelial or adventitial fibrosis develops (Fajardo, 2005). In these vessels, the media is replaced by 'hyaline' acellular acidophilic matrix. In medium-sized arteries, intimal fibrosis is more common (Sams, 1965; Fajardo, 2005). In these lesions, collagen and myofibroblasts are present and can result in narrowing of the vessel lumen. Larger arteries are less often affected by fibrosis, while intimal and medial fibrosis is an occasional feature of radiation damage in small veins and periarterial fibrosis is seen as a consequence of radiation exposure.

Radiation-induced fibrosis is driven by inflammation and tissue remodelling through complex processes involving various cellular mediators and cytokines. It represents a delayed manifestation of a deregulated wound healing response to radiation injury where processes that would normally serve to terminate fibrogenesis are not appropriately switched off (Denham and Hauer-Jensen, 2002; Dormand et al, 2005).

The pathophysiology of radiation-induced fibrosis is not completely established, and the precise sequence of events, the cellular participants and signalling mediators driving the process are still being defined. Nevertheless, it is established that significant vascular remodelling generally precedes development of fibrosis and radiation-induced endothelial loss and endothelial dysfunction are now considered to be major triggers of normal tissue damage leading to fibrosis (Wang et al, 2007). Injury to the endothelium leads to oedema and fibrin deposition, which may remain unresolved by an inhibited plasminogen activator system (Fajardo and Berthrong, 1978; Hageman et al, 2005). Endothelial loss could contribute to deregulated fibroblast and smooth muscle cell growth and matrix protein secretion (Herold et al, 1999). Irradiated endothelial cells produce TNFα and platelet derived growth factor (PDGF), which are potent activators of fibroblast and smooth muscle cell proliferation and collagen production (Li et al, 2007). Down-regulated expression of endothelial nitric oxide synthase and nitric oxide production by radiation also release inhibitory effects on smooth muscle cell proliferation (Sugihara et al, 1999). Irradiated endothelial cells enhance the fibrogenic phenotype of fibroblasts and smooth muscle cells by inducing production of connective tissue growth factor (CTGF) and collagen (Milliat et al, 2006). CTGF is a major mediator of fibrosis, downstream of pro-fibrotic cytokines including transforming growth factor beta (TGF-β) and, once expressed, triggers further cellular mediators of fibrosis and production of matrix (Daniels et al, 2009; Gervaz et al, 2009).

Activation of the coagulation cascade plays a distinctive role in the early stages of radiation-induced vascular damage and could be a trigger for subsequent fibrotic responses (Wang et al, 2007). Thrombomodulin down-regulation (Zhou et al, 1992) leads to elevated levels of thrombin, which in addition to participating in clotting also acts as a potent activator of various cell responses through binding to cell surface proteinase activated receptors (PARs) (Martorell et al, 2008). Thrombin directly stimulates and recruits fibroblasts, myofibroblasts and smooth muscle cells, thereby enhancing fibro-proliferative processes. In addition, thrombin stimulates collagen production and secretion.

TGF-β is, however, by far the most widely studied and most potent known regulator of fibrosis. In radiation fibrosis it acts through promoting the proliferation of fibroblasts and smooth muscle cells while inhibiting the growth of endothelial and epithelial cell types (Martin et al, 2000). TGF-β also stimulates cells to increase synthesis of matrix proteins and decrease production of matrix degrading proteins. It is secreted as a latent complex that requires activation in order to bind to its receptors (eg Massagué, 1996). Radiation activates the latent form of the TGF-β protein (Barcellos-Hoff et al, 1994) in addition to up-regulating its expression, not only in endothelial cells but also by many cell types including fibroblasts, myofibroblasts and smooth muscle cells (Martin et al, 2000). Activated TGF-β binds to type II receptors (TGF-β-IIR) and then forms a complex with TGF-β type I receptors (TGF-β-IR) (Moustakas and Heldin, 2009). Downstream signalling is then mediated through Smad proteins that translocate to the nucleus where they act as transcription factors. In particular, Smad2 and Smad3 proteins activate the expression of genes that inhibit extracellular matrix degradation such as plasminogen activator inhibitor 1 (PAI-1) and stimulate pro-fibrotic genes such as CTGF and collagens. In non-vascular sites maintenance of fibrosis is thought to be dependent on the auto-induction activities of TGF-β and CTGF, which remain elevated in fibrotic tissues (Haydon and Vozenin-Brotons, 2007). More recently, signalling through the small family of Rho-GTPases and associated Rho kinase has been implicated in the initiation and maintenance of radiation-induced fibrosis and shown to transduce signalling by TGF-β (Haydon et al, 2005). Rho signalling is also

directly activated by radiation (Gabrys et al, 2007) and is emerging as an alternative pathway for regulating CTGF in fibrosis (Gervaz et al, 2009). Both TGF-β and Rho are now considered as potential targets for anti-fibrotic strategies.

6.3 Effects of Radiation in the Heart Microvascular Network

As noted previously, damage to normal tissue blood vessels is a common feature of therapeutic irradiation, with endothelial cells described as being particularly sensitive (Fajardo, 1989). In normal tissues and at clinically relevant doses, acute vascular damage can be evident within hours of irradiation (Krishnan et al, 1988; Baker and Krochak, 1989; Roth et al, 1999). Apoptosis and increased permeability, oedema and lymphocyte infiltration are common features of such damage, whereas delayed vascular effects such as capillary collapse, scarring and fibrosis are often evident weeks or months after irradiation.

Damage to the microvascular network in the heart by radiation was demonstrated many years ago. Using the New Zealand white rabbit model, Luis Fajardo and colleagues showed that damage to the myocardium develops in several stages involving damage to small- and medium-sized vessels (Fajardo and Stewart, 1970, 1973; Fajardo and Brown, 1973). In this model, acute inflammation with neutrophil infiltration was evident within a few hours of irradiation. Electron microscopy analysis showed endothelial damage and evidence of thrombosis within the myocardium two days after irradiation. At this time, endothelial cells appeared swollen and vacuolarised and had irregular cytoplasmic projections into the lumen. Thrombi and platelets were also present obstructing the microvascular networks (Fajardo and Stewart, 1973). Endothelial cells showed increased proliferation, although this did not prevent an eventual regression of capillary networks. Fibrosis and myocardial degeneration were evident at later stages.

Studies by Lauk and colleagues showed that in the rat heart irradiation caused myocardial degeneration associated with focal loss of the endothelial marker enzyme alkaline phosphatase (AP) (Lauk, 1987). The significance of AP activity and its radiation-induced loss is not known. However, AP is associated with processes such as proliferation and maintenance of blood flow within the microvascular system and is thought to be a marker of a functional endothelium. A detailed study by Schultz-Hector and Balz (1994) demonstrated the progressive nature of myocardial degeneration and clinical heart failure in rats receiving a 20 Gy single dose to the heart. The investigators evaluated the progressive loss of AP in two strains of rat in which radiation-induced cardiomyopathy developed with different latencies. In these models, both latency and severity of radiation-induced microvascular damage correlated with initial levels of AP expression. In Wistar rats, with lower pre-irradiation AP levels, loss of enzyme activity was detectable earlier (within 25 days) than in Sprague-Dawley rats (60 days), which had more pronounced initial levels of AP (Schultz-Hector and Balz, 1994). Loss of AP activity at the early time point was associated with endothelial swelling observed at the ultrastructural level, while adjacent cardiomyocytes appeared normal. The extent of enzyme-negative foci increased with time after irradiation in both strains and these foci were associated with increased leukocyte and platelet adhesion. Maximal enzyme loss was evident at 90 days in the Wistar rat and progressed to rapture of capillaries and red blood cell extravasation, thickening of the basal membrane and an increased presence of fibroblasts. Gradually, small foci devoid of capillaries appeared and these expanded in large areas of the myocardium. Proliferation of

microvascular endothelial cells was also evident but capillaries regressed possibly because of radiation effects on mitotic cells (Lauk and Trott, 1990). As capillary density decreased, progressively signs of cardiomyocyte degeneration and necrosis became evident, which were always associated with ischaemic foci negative for AP. In Sprague-Dawley rats, similar changes were observed but only after 130 days and these were less severe in nature. Heart irradiation with lower (but still high) doses (15 Gy) did not result in clinical failure and allowed animals to be evaluated for up to two years, demonstrating that AP-negative areas persisted for prolonged periods after radiation (Schultz-Hector, 1993). Whole body but not local thoracic irradiation at 10 Gy has been observed to cause microvascular damage leading to cardiac dysfunction (Baker et al, 2009).

These studies illustrate the progressive and persistent nature of the damage caused by radiation to the heart microvascular system. They also highlight the pivotal role of the initial radiation injury to the microvascular network in the heart in subsequent myocardial loss and cardiac failure. This sequence of events is further supported by evidence that radiation does not cause direct damage to the non-dividing cardiomyocytes. Microvascular injury is therefore thought to lead to a progressive reduction in functional capillaries, causing ischaemia and consequent myocardial degeneration.

6.4 Pre-existing Risk Factors

The risk factors for atherosclerosis have already been described, and include age, male gender, smoking, diabetes, hypertension and hyperlipidaemia. Whilst most of the effects of irradiation on the vessel wall appear to be local, whole body irradiation can increase plasma lipoprotein lipase activity, hepatic cholesterol and triglycerides levels, and lipid peroxidation products (Pote et al, 2006) in some studies, which may be pro-atherogenic. In contrast, in other studies cholesterol levels in irradiated mice were not significantly different from that in age-matched controls, and markers of systemic inflammation (soluble ICAM-1, soluble VCAM-1 and CRP) were not elevated (Stewart et al, 2006). Endovascular irradiation can increase oxidised LDL in the plasma (thiobarbituric acid-reactive substances) and in arterial tissue, associated with increased atherosclerosis, suggesting that some of the pro-atherosclerotic effect of irradiation is due to free-radical-induced lipid oxidation (Leborgne et al, 2005). Finally, the atomic bomb survivors show an increased incidence of hypertension, particularly in those with exposures above 2 Sv (Sasaki et al, 2002; Yamada et al, 2004), and elevated levels of serum cholesterol (Wong et al, 1999), suggesting that generalised, systemic factors may promote atherosclerosis in these patients.

6.5 Dose–response for Radiation Effects

6.5.1 Experimental *in vitro* studies

Adhesion of leukocytes to the vascular endothelium is an essential step in the inflammatory process and is mediated via increased expresion by endothelial cells (EC) of selectins and adhesion molecules such as E-selectin and ICAM-1. E-selectin was up-regulated in a time-dependent fashion by doses of as little as 0.5 Gy in human vein endothelial cells (HUVEC) via transcriptional regulation (Hallahan et al, 1995). This expression was independent of cytokines, and is related to increased binding of nuclear proteins from

irradiated ECs to the NFκB binding site of the E-selectin promoter: knockout of this binding site eliminated the X-ray up-regulation of E-selectin (Hallahan et al, 1995). Both E-selectin and ICAM-1 were up-regulated in human ECs with X-ray doses of between 1 and 5 Gy, although levels of other adhesion molecules (VCAM and P-selectin) were unaffected (Hallahan et al, 1996; Quarmby et al, 2000). Induction of E-selectin and ICAM-1 occurred immediately and was independent of radiation-induced cytokine [interleukin 1 (IL-1) and tumour necrosis factor α (TNFα)] production. However, whilst E-selectin could be induced by doses down to 0.5 Gy and the effect was transitory (levels had returned to baseline by 20 hours), ICAM-1 induction required a dose of 5 Gy and expression still persisted at 48 hours (Hallahan et al, 1996). High doses have further been observed to lead to changes persisting for 10 days (Gaugler et al, 1997).

Quarmby et al (2000) confirmed the up-regulation of ICAM-1 but at the somewhat lower dose of 2.5 Gy and also observed increased expression of PECAM-1/CD31 at doses of 5 Gy and above. Irradiation of 10 Gy can lead to long-term (21 day) elevation of PECAM-1 (Gaugler et al, 2004). In human pulmonary microvascular endothelial cells (HMVEC-L), ICAM-1 expression was raised within 24 hours of 2 Gy X-irradiation (Hallahan and Virudachalam, 1997a). In cultured human epithelial skin cells exposed to 5 Gy X-rays there was up-regulation of ICAM-1, VCAM-1 and E-selectin cell surface expression within 24 hours, although levels of PECAM-1/CD31 were unchanged (Heckman et al, 1998). However, exposure of a transformed human bone marrow EC line (TrHBMEC) to 2 Gy cobalt-60 gamma rays resulted in increased expression of ICAM-1 but no change in VCAM-1, E-selectin and PECAM-1 (Gaugler et al, 1998). This study also reported up-regulation of certain cytokines, in particular IL-6, IL-8, IL-11, IL-1α, G-CSF and GM-CSF, but no change in levels of other pro-inflammatory cytokines, such as TNFα or LIF. Somewhat at odds with this, Woloschak et al (1990) demonstrated up-regulation of the pro-inflammatory cytokine IL-1 in Syrian hamster embryo cells *in vitro* following irradiation by 0.75 Gy X-rays, 0.9 Gy gamma rays or 0.21 Gy fission neutrons.

In contrast to the above, doses in the range 0.3–0.7 Gy are associated with reduced adhesion of human peripheral blood mononuclear cells (PBMC) to EC, this being most likely associated with the shedding of L-selectin from the surface of peripheral T-cells (Kern et al, 2000). Further studies in the same dose range with the same cell type have shown that this is accompanied by a decrease in expression of E-selectin and PBMC adhesion to EC, accompanied by an increase in levels of the pro-inflammatory cytokines TGF-β and IL-6 (Roedel et al, 2002). A study using WHT/Ht mouse peritoneal macrophage cells indicated that 2 Gy X-rays down-regulated IL-1β and IL-6, whereas 0.1 Gy increased IL-6 expression but had no influence on IL-1β expression (Hosoi et al, 2001). Thus, in contrast to high dose irradiation, acute doses in the 0.1–1 Gy range may result in down-regulation of the adhesion of leukocytes to the endothelium and thus have an anti-inflammatory effect. One mechanism influencing the response to radiation at different doses may be the nitric oxide pathway in stimulated macrophages, since NO is known to play a central role in inflammation. When macrophages were stimulated with lipopolysaccharide (LPS) and interferon-gamma (IFNγ), NO production was suppressed at X-ray doses up to 1.25 Gy, but returned to normal and increased at higher doses (Hildebrandt et al, 1998). Since levels of TNFα were unaffected, it was concluded that radiation was having a direct effect on the inducible nitric oxide synthase (iNOS) pathway through post-transcriptional or post-translational regulation of iNOS (Hildebrandt et al, 1998).

6.5.2 Experimental *in vivo* (animal) studies

Generally, animal studies confirm that acute doses of around 2 Gy and above are associated with increased expression of a variety of cell adhesion molecules in endothelial tissue (Hallahan and Virudachalam, 1997b; Schultz-Hector and Trott, 2007). Thoracic irradiation of C3H mice by 2 Gy X-rays resulted in increased expression of E-selectin, P-selectin (another pro-inflammatory factor promoting leukocyte rolling) and ICAM-1, but expression differed in different tissues (Hallahan and Virudachalam, 1997b). Thus ICAM-1 was primarily expressed in the endothelium of the microvasculature, E-selectin was expressed primarily in the endothelium of the larger blood vessels, and P-selectin primarily in the endothelium of Weibel-Palade bodies of the endothelium, never in the microvasculature. This selective expression may explain some of the inconsistencies between *in vitro* studies. The increased permeability of ECs can also lead to increased accumulation of lipids and initiation of atherogenic changes in the presence of hypercholesterolaemia in C57BL/6 mice after doses of 8 Gy X-rays (Tribble et al, 1999).

As observed *in vitro*, acute doses in the range 0.1–1 Gy can result in down-regulation of the adhesion of leukocytes to EC and thus may have an anti-inflammatory effect. For example, whole body irradiation of rats with 0.1, 0.3 and 0.6 Gy 6 MeV photons had no effect on ICAM-1 expression but was found to inhibit leukocyte adhesion following challenge with LPS (Arenas et al, 2006). Hildebrandt et al (1998) extended their *in vitro* work by examining radiation effects on the nitric oxide pathway *in vivo* using the murine (BALB/C) chronic granulomatous air pouch system and were able to confirm that low dose irradiation modulates the production of NO. Such results provide mechanistic support for the clinical efficacy of low to medium dose radiotherapy for the treatment of inflammatory conditions (Trott, 1994) and the suggestion that different radiobiological mechanisms are involved at the higher and lower ends of the dose spectrum (Hildebrandt et al, 1998).

To summarise, radiation can modulate inflammatory reactions over a range of doses, at doses above about 0.5 Gy a generally pro-inflammatory response is observed. Some data suggest that at lower dose levels an anti-inflammatory response may predominate. However, it is clear that we have an as yet incomplete understanding of the effects of radiation on inflammation. Thus there is a need for research into the impact of radiation at different dose levels on the factors that mediate inflammatory responses and their net impact on radiation-associated diseases.

6.6 Conclusions

The underlying causes of circulatory diseases are multifactorial. Many of the postulated mechanisms for the development of circulatory diseases, in particular atherosclerosis (eg inflammation), are also seen as consequences of radiation exposure. Furthermore, radiation exposure may not only act as an independent risk factor for the development of circulatory diseases, but may also influence the more conventional, well-established risk factors. There still remain no firm biological causal links between radiation exposure and circulatory diseases, and further studies are required to demonstrate direct associations between radiation exposure and surrogates for cardiovascular disease.

6.7 Summary

1 The development of circulatory diseases is multifactorial.

2 Many of the underlying contributory mechanisms associated with the development of circulatory disease, particularly atherosclerosis, are also associated with radiation exposure.

3 Primary atherosclerosis is associated with DNA damage.

4 Radiation-induced induction or potentiation of atherosclerosis is associated with increased DNA damage on the background of extensive DNA damage, and/or exacerbation of pre-existing risk factors.

5 There is minimal and inconsistent evidence to suggest that primary atherosclerosis arises through cell transformation.

6 In contrast, advanced atherosclerosis is associated with cell senescence, cell death, mitochondrial dysfunction, thrombosis and inflammation.

7 Cell senescence, cell death, thrombosis, mitochondrial dysfunction, inflammation and fibrosis are all potentiated by irradiation, and all promote atherosclerosis. However, the contribution of these processes to radiation-associated circulatory disease, particularly at low dose levels, is not yet clear.

7 Consideration of Models for Estimation of Cardiovascular Disease Risk in Exposed Human Populations

Is there a Stochastic Component in Radiation-induced Atherosclerosis? – Implications for Risk at Low Doses

Detrimental responses arising from radiation exposure are regarded as deterministic (now referred to by the ICRP as tissue or organ reactions) if they are believed to occur only above a threshold dose. Such responses are generally thought to result from the killing, senescence or apoptosis of a critical number and/or type of cell. Such cell death is almost certainly involved in damage to the circulatory system following high and very high dose radiation exposure. In contrast, stochastic responses are believed to occur at both high and low doses and are hypothesised to result from persisting changes (including mutations) in individual cells which are then subject to clonal expansion. For example, cancers arising after radiation exposure are regarded as stochastic in origin and the implication is that there is always some (albeit small) risk even at low doses. For radiological protection purposes, therefore, a linear no-threshold dose–response is assumed for stochastic effects, as a prudent and pragmatic judgement. Epidemiological evidence for this is scarce but there is at least one case where the evidence is compelling, namely the induction of lung cancer by radon (AGIR, 2009).

We have discussed (in Chapter 6) the evidence that smooth muscle cells (SMC) in atherosclerotic plaque are clonal. The question therefore arises as to whether the occurrence of atherosclerotic plaques could be a stochastic process following radiation exposure. If it were, then circulatory disease would need to be considered as a component of risk following occupational and environmental exposure to radiation. The question that needs to be addressed is whether there are persisting changes in SMC or other cells (eg endothelial cells or monocytes) that are causal in the development of plaques and whether such changes can be induced by radiation. Although SMC in plaques frequently show clonality, this does not necessarily imply that clonal genetic changes are causal in plaque formation. Indeed, the clonality of plaques may well have a developmental origin (Schwartz and Murry, 1998). The question is whether clonal changes determine the pathological nature of the plaque.

Examination of SMC from spontaneous plaques has revealed many changes that distinguish them from normal SMC: loss of heterozygosity, increase in micronuclei and microsatellite mutations, and other evidence of genomic instability (Hatzistamou et al, 1996; Andreassi and Botto, 2003). While all of these changes are thought normally to be a consequence of the active oxygen species that occur concomitantly with the inflammation associated with developing plaques, they could in principle also be

inducible by radiation. Such changes are essentially markers that DNA damage has occurred; they may or may not in themselves have a causal role in plaque formation.

There are, however, two types of change that demand more serious consideration. The first is that SMCs isolated from plaque show persisting changes in phenotype including their outgrowth characteristics and inability to respond to certain muscle relaxants (Pickering et al, 1992; Jones et al, 1996). Such phenotypic changes imply changes in the genome and/or changes in gene expression. Indeed, changes in gene expression are widespread in plaque SMC (Mulvihill et al, 2004) and include overexpression of myc (Parkes et al, 1991) and p53 (Iacopetta et al, 1995). The fact that they are not random is consistent with (but does not validate) the idea that they contribute to the phenotype of plaque SMC. We know that radiation can cause changes in gene expression, but in general such changes are fragile, with few consistent variations seen in different systems (Amundson, 2008). The fact that such changes are usually transitory, lasting generally no more than a few days (Amundson, 2008), suggests that they are probably not involved in possible late chronic effects (in particular cardiovascular disease) of low dose exposure.

The second significant change is a transfectable property that can be isolated from the DNA of plaque but not normal SMC and which confers morphological changes in cultured mammalian cells and gives rise to tumours in nude mice. This work (Penn et al, 1986, 1991; Penn, 1990) has been carried out in several systems in this laboratory and the genes myc, p53 and c-Ki ras have all been ruled out as candidates for the transfectable activity (Parkes et al, 1991; Iacopetta et al, 1995). There are, however, no reports concerning a transfectable factor from other laboratories, either in confirmation or in contradiction. Furthermore, there is no evidence as to whether radiation can induce the transfectable factor activity, although in one system it has been induced by a chemical mutagen (Penn et al, 1991).

Taking these results together, it is possible to argue that changes in genetic material (mutational and/or epigenetic) might take place when normal SMC develop into plaque SMC. If these changes were essential for plaque formation then it is reasonable to expect that they would be induced by radiation, a known and powerful mutagen. Supposing, for the sake of argument, that the above supposition were correct, what would be the consequences for radiation risk at doses relevant to radiological protection? Logically, the induction of atherosclerosis would need to be included in the detriment ascribed to radiation exposure even at low doses. However, as discussed in Chapter 6, current consensus holds that mutational mechanisms do not drive atheroscleosis.

Against this, it may be argued that there is so much DNA and other damage due to inflammatory processes in developing plaques that the small amount that could come from environmental or occupational radiation exposure would be insignificant. However, that this argument is in need of modification is suggested by the case of radon and lung cancer. The lungs of smokers are chronically inflamed as a result of continuous bombardment with toxic substances including mutagens and promoting agents. These lead to a lung cancer risk around ten times that of non-smokers. Nevertheless, smokers exposed to radon have an even greater risk of lung cancer, and this is true even at environmental levels of radon (Darby et al, 2005a; Krewski et al, 2005; BEIR, 1999; AGIR, 2009); the increase is consistent with a submultiplicative interaction between radon progeny and cigarette smoke (BEIR VI Committee, 1999). It is apparent that radiation and smoking act to some extent synergistically in the generation of lung cancer and that the radiation dose–response is not significantly different from linear. It should

therefore be considered possible that radiation and inflammation in early atherosclerosis may also act synergistically in the development of plaques. It should be borne in mind that cigarette smoke is a potent mutagen, and it is more likely that mutational damage *per se* rather than inflammation (which is a consequence of this mutational damage) is the cause of lung cancer.

As discussed in Chapter 6, there is abundant evidence that mutational mechanisms are unlikely to play a large role in cardiovascular disease. Apart from mutation the main other documented effects of radiation exposure are cell killing and senescence (eg Sermsathanasawadi et al, 2009). It may be useful to consider in what ways induced sterilisation or senescence of critical cell populations (endothelial cells, T-lymphocytes, monocytes, macrophages and SMC) could increase cardiovascular risk. Given that, in general terms, one main function of endothelial cells is as signallers of damage, radiation-induced sterilisation or senescence would probably reduce risk, although senescent or dead cells would, however transitorily, increase the permeability of the arterial wall (until the surrounding cells regrew to refill the gap in the arterial lining). It is also the case that senescence or apoptosis of cells in the cap of atherosclerotic plaques will serve to weaken the plaque and increase the probability of plaque rupture which may then promote clotting in important vessels leading to obstruction. In this sense, senescence and apoptosis would be risk enhancing. As discussed in Chapter 6, SMC are important in later stages of lesion development, but perhaps not in the early stages of lesion development. This leaves monocytes and T-lymphocytes as possible candidate cell species for radiation action.

A recently developed mathematical model of the cardiovascular system proposed a novel mechanism based on radiation-induced monocyte killing in the intima and a resultant increase in monocyte chemo-attractant protein 1 (MCP-1) that binds to them (Little et al, 2009b). As well as predicting radiation risks consistent with what is seen in nuclear workers, the changes in MCP-1 with dietary cholesterol predicted by the model are also consistent with experimental and epidemiological data (Little et al, 2009b). The model predicts that risks would vary almost linearly with dose over the range 0–4 Gy (Little et al, 2009b). However, this novel biological mechanism has yet to be experimentally tested.

The role of inflammation, thrombosis and fibrosis and the signalling of these processes in atherogenic disease is well established. The radiation dose–response relationships for these endpoints are not well established, particularly in the low dose region. Pro- and anti-inflammatory effects of radiation have been described, with most suggestions for anti-inflammatory effects being associated with low dose exposures. Certainly at high doses, radiation can increase inflammation, fibrosis and thrombosis, and so radiation induction of these processes would be viewed as risk enhancing. To be certain of the effect of low radiation doses on circulatory disease good dose–response data for these processes will be required. Classically though these are viewed as high dose exposure phenomena and their relevance at low doses is not clear. Nonetheless, it is quite possible that radiation may act via transient (or possibly longer lasting) changes in vascular adhesion – for example, mediated by up-regulation of VCAM-1, ICAM-1 and E- and P-selectin. As discussed in Chapter 6, there is evidence for transient radiation-associated changes in these inflammatory markers, although it is not clear at what levels of dose these operate, and at low doses below 1 Gy there is some evidence (albeit inconsistent) for changes in the direction of the dose–response for these and other (anti-inflammatory) cytokines. At high doses, changes in inflammatory markers can persist for days. Even transient changes in arterial adhesion could result in a mechanism that would be additive in small increments of dose.

What conclusions then can we draw from the evidence in this chapter? Were the involvement of a stochastic process to be demonstrated convincingly, it would have significant implications with respect to radiation risk coefficients. However, atherosclerotic disease is a multifunctional disorder and all aspects of its biology need to be considered in relation to causal factors. We do not consider that the available evidence justifies consideration of a stochastic component as being established, although it remains as a possibility. Clearly, further work is needed to establish whether or not radiation can induce transformation of SMC to a plaque-type phenotype, whether this induction is a stochastic process, and whether it plays a significant role in atherogenic development. Similarly, more information is required on the lower range dose–response for the processes implicated in atherosclerotic disease such as inflammation, thrombosis and fibrosis.

8 Conclusions and Recommendations

8.1 Conclusions

1 Radiation exposure of the heart and circulatory system can occur in several contexts and at several levels. The entire population will be exposed to the UK average effective dose of 2.7 mSv y^{-1}, although not all of this will be delivered to the circulatory system. Radiation workers may receive higher doses and those receiving medical diagnostics, some medical interventional radiological proceedures and, particularly, radiotherapy may receive doses to the circulatory system, or parts of it, up to the level of several gray (absorbed dose). Assuming that radiation acts to increase spontaneous disease incidence multiplicatively, and the analysis in Chapter 4 indicates that the available data suggest an excess relative risk of 0.09 Sv^{-1} (95% CI 0.07, 0.12), even small relative risks due to radiation could have a major impact on levels of radiation-attributable disease as circulatory diseases are already common in the population. This is therefore an issue of potential importance in radiation protection.

2 Evidence from radiotherapy follow-up studies and from experimental animal models indicates that irradiation at high and very high doses increases circulatory disease risk. Radiation doses to the heart in radiotherapy studies are not always known precisely, but heart doses in excess of 5 Gy are likely. Precise estimation of radiation risk is often complicated by the use of known cardiotoxic drugs (such as anthracyclins) as an adjunct to radiotherapy. Some medical irradiation studies, particularly those following up female breast cancer radiotherapy patients, hold some prospect of providing epidemiological evidence of risk at slightly lower doses. Initial indications from these studies suggest that there is excess risk of cardiovascular disease (Darby et al, 2003) but organ dosimetry is complex in these situations, and is being evaluated (Taylor et al, 2007, 2008, 2009a,b). Studies following radiotherapy for childhood cancer with high quality organ dosimetry are only just starting to be published (Mulrooney et al, 2009; Tukenova et al, 2010) and further studies post-childhood cancer and breast cancer can be expected soon. In general, in these radiotherapy studies excess risk is statistically significant only at very high dose levels of 15 Gy and above (Mulrooney et al, 2009; Tukenova et al, 2010).

3 A statistically significant increase in the risk of certain circulatory diseases (notably, stroke, heart disease and specifically ischaemic heart disease) has been detected in low and moderate dose (below 5 Gy) epidemiological studies, notably the atomic bomb survivor studies and nuclear worker studies. While heterogeneity between the studies is considerable, statistically significant excess risk can be detected at around 0.5 Gy. However, it is well known that there are many contributory risk factors to circulatory disease (eg cigarette smoking, diet and alcohol consumption) which may confound these studies. Only two studies are judged to control adequately for major lifestyle confounders such as smoking and alcohol consumption. While

these studies provide evidence of association they do not provide evidence for radiation being causal for these diseases.

4 In broad terms, evidence for radiation causality of, or convincingly strong association with, circulatory disease below doses of 0.5 Gy is considered to be very unlikely to emerge from human population studies in the near future. Insights from mechanistic experimental studies might eventually provide the required weight of evidence of causality at low radiation doses.

5 The processes that contribute to the development of radiation-associated atherosclerotic disease are not fully understood. Some of the steps promoting the development, destabilisation and rupture of atherosclerotic plaques (which trigger disease by initiating clot formation in important vessels) are known to be affected by radiation – for example, senescence, cell killing and modulation of cell proliferation. The involvement of stochastic genetic events or other events that might result in a linear dose–response relationship cannot be excluded but evidence of their involvement is weak. Smooth muscle cells in atherosclerotic plaques are phenotypically different from those of normal arterial walls. While many of these differences persist in culture the evidence suggests that they are due to changes in gene expression rather than to mutations. Evidence that a cancer-like phenotype can be transformed from plaque DNA has not been confirmed and there is no plausible evidence for any other type of mutation.

6 Evidence for a mutational component to circulatory diseases would not be the only factor consistent with a linear no-threshold dose–response curve. A linear dose–response model attributing atherosclerosis to the killing of monocytes by fractionated low radiation exposures recently has been proposed (Little et al, 2009b). Nevertheless, at present convincing evidence that might justify a linear no-threshold dose–response curve is absent. Until there is clarity on the role and mechanisms of radiation action in causing circulatory disease (supporting or refuting a stochastic mechanism of action), the conventional ICRP radiation weighting factors (w_R) and the radiation protection quantities of sievert, equivalent dose and effective dose, which apply only to recognised stochastic effects such as cancer induction and hereditary effects, should not be used in preference to gray (Gy) and RBE-weighted Gy for these tissue reactions.

7 There is currently little evidence to justify the inclusion of circulatory disease in calculation of radiation detriment at doses of 0.5 Gy and below. However, the AGIR concludes that it would be appropriate to incorporate circulatory disease risks into specific retrospective health risk assessments for individuals exposed to doses above 0.5 Gy.

8.2 Recommendations

1 There are multiple contributory risk factors for circulatory disease, including genetic, environmental and lifestyle factors. The HPA should continue to advocate avoidance of these risk factors where possible to minimise the incidence of such diseases in the population and to reduce the impact of any synergies with radiation.

2 Given the evidence for radiation of critical vasculature at high and very high doses leading to health problems, notably heart disease and stroke, the AGIR recommends that the HPA encourages medical practitioners who deliver radiation for either therapeutic or diagnostic purposes and their professional associations to examine current practice with a view to minimising radiation of critical vasculature while maintaining effective clinical outcome and to consider a follow-up programme for exposed patients to improve estimation of risk.

3 The uncertainties on radiation causality of atherosclerotic disease are likely to be reduced only when an improved understanding of the impact of radiation at different dose levels on the development, destabilisation and rupture of atherosclerotic plaques is available. Many processes such as cell senescence, cell death, thrombosis, mitochondrial dysfunction, inflammation and fibrosis are implicated in atherogenesis and are also induced by radiation exposure. The AGIR notes that atherogenesis in rodent models and humans has important differences as well as significant similarities. The AGIR recommends that the HPA develops research to improve mechanistic understanding of the impact of radiation on atherosclerotic plaque formation and destabilisation, where possible using human systems.

4 Biomarkers of circulatory disease risk are, in general, not well developed; any research that might identify such markers should be encouraged by the HPA. Such biomarkers would be of value in establishing whether radiation is causal for circulatory disease. These biomarkers could be biochemical, cellular or physiological. Imaging methods to assess vascular function might be especially useful as non-invasive techniques.

5 The AGIR recommends that in retrospective health risk assessments of individuals exposed to doses of 0.5 Gy and above, consideration of circulatory disease risk should be included.

6 In anticipation of the possible future emergence of evidence for radiation causality of circulatory disease below 0.5 Gy, the AGIR recommends that the HPA considers the implications of such evidence for radiation protection.

7 Further follow-up of the atomic bomb survivors and various occupationally exposed groups (eg nuclear workers and the Chernobyl liquidators) is strongly recommended, and is likely to be increasingly informative of risks at medium and low doses.

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Appendix
Epidemiological Study Designs

A1 Types of study

Epidemiology is the study of the distribution and determinants of disease in human populations (MacMahon and Trichopoulos, 1996). It is by its nature observational rather than experimental. In contrast to randomised controlled trials (which are largely experimental in design), in epidemiological studies there is always the possibility that biases or confounding factors of various sorts may give rise to spurious results, as discussed in more detail below. A well-designed study should attempt to minimise these. A good investigator will design a study to have adequate statistical power; this is discussed in greater detail by UNSCEAR (Section I.B: UNSCEAR, 2006). Epidemiological studies are commonly of two types: the cohort study and the case–control study.

In a 'cohort study', a defined population (preferably with a wide range of radiation exposures) is followed forward in time to examine the occurrence of many possible health endpoints. Such a study can be performed either prospectively, by following a current cohort into the future, or retrospectively, by using registers to construct a cohort of persons alive at some time in the past, and then following it forward, possibly to the current time and beyond. The Life Span Study (LSS) of the survivors of the atomic bombings in Japan is an example of a cohort study, partly retrospective and partly prospective in nature. The LSS data were assembled in the late 1950s using questions posed in the Japanese national census of October 1950 to ascertain those persons who were in either Hiroshima or Nagasaki at the time of the atomic bombings. This cohort was then followed forward in time, and is still being followed up, for mortality due to all causes (Pierce et al, 1996; Preston et al, 2003, 2004; Shimizu et al, 2010), cancer incidence (Preston et al, 1994, 2007) and various other endpoints (Otake et al, 1990; Otake and Schull, 1993; Wong et al, 1993; Yamada et al, 2004). A 'correlation study' is a particular type of cohort study that is based on data averaged over groups, and in particular uses data grouped on exposure. In a 'randomised controlled trial (RCT)', people are assigned at random to various groups before planned exposure to radiation – eg radiotherapy treatment (Fisher et al, 1985), and these groups are then followed up to assess their response to the treatment over some defined period. An RCT may be regarded as a special form of cohort study; however, its essentially experimental design, as opposed to the more observational design of most cohort studies, should be noted.

In a 'case–control study', data on persons with some specified disease (eg some class of cancers) are assembled (the 'cases') together with data on a suitably matched (eg by age and sex) set of persons otherwise similar to these cases but without the disease (the 'controls'). These two groups are then compared to assess differences in the distribution of a number of exposure variables. The advantage of a case–control study is that detailed histories of radiation exposure and other information (eg history of smoking), which may be difficult to collect for a cohort, can be collected relatively easily for the specific cases and controls. The International Radiation Study of Cervical Cancer Patients (IRSCCP) is an example of

a series of nested case–control studies of the occurrence of a second primary cancer in a cohort of women followed after treatment for a first primary cancer of the cervix (Boice et al, 1987, 1988, 1989).

Another form of study, not so frequently used, is the 'case–cohort' or 'case–base' study (Prentice, 1986), in which information is collected on all cases with a certain disease status (eg cancer) as well as on a sample of persons from the underlying cohort, sampled without regard to their disease status. This type of study is particularly useful when a number of different endpoints are of interest, because it is possible to reuse the cohort sample for each disease endpoint under consideration. This study design was used in an early analysis of the IRSCCP (Hutchison, 1968). Other, more novel designs, which generalise the above, have recently been proposed (Langholz and Goldstein, 2001).

An RCT, if the randomisation is conducted properly, should not be subject to any bias, and is generally regarded as the epidemiological 'gold standard'. The case–control study is prone to more biases (eg recall bias and investigation bias — see below) than the cohort study, and for this reason cohort studies are regarded as the next most reliable type of study after the RCT.

A2 Difficulties in drawing conclusions on cause–effect relationships from observational studies

'Bias' in a study may be defined as any process at any stage in the conduct of the study that tends to produce results or conclusions that differ systematically from the truth.

One sort of bias is 'follow-up bias', which arises when there is a lack of follow-up information – for example, if persons have, unknown to the investigator, migrated outside of the study area, so that their health status cannot be reported. In this instance, they still apparently contribute to the number of person-years (PY) of follow-up in the study, but in reality there is no chance of observing any detrimental effect to their health, making them appear 'effectively immortal'. Unless corrected for, by censoring members of the study cohort (ie stopping their contribution to the total number of person-years) when they are lost to follow-up, estimates of disease risks will generally be biased downwards and therefore be underestimates of the true risk. This form of bias applies equally to cohort studies and case–control studies. It is sometimes supposed that case–control studies are immune to this bias, but this is not so; case and control selection will be biased if certain members of the full cohort are not available to be selected.

Related to follow-up bias is 'ascertainment bias', also sometimes known as 'selection bias', which arises when there is variation in ascertainment of disease status, perhaps correlated with exposure variables. For this reason, much the strongest studies are those that rely upon independently maintained registers of disease and health status, eg the mortality and cancer incidence registers maintained in many developed countries. As an example, certain tumours, such as those of the thyroid, are notoriously difficult to detect, so that the recorded incidence of thyroid cancer in a cohort will very much depend on the diligence with which clinical examinations have been conducted in the underlying cohort. In the LSS cohort of the survivors of the atomic bombings, the ascertainment of thyroid tumours is better in the higher dose groups, because many people in these groups are subject to biennial screening (Preston et al, 2007), as

they are also members of the Adult Health Study (AHS), a subcohort of the LSS. Unless corrected for, this ascertainment bias, which is correlated with dose, would bias the slope of the dose–response curve upwards; however, in this case the ascertainment bias can be corrected for by stratification of the cohort according to membership in the AHS, and conducting a suitably adjusted analysis (Preston et al, 2007). Another example of such bias occurred in a study of workers involved in the recovery from the Chernobyl accident, for whom a statistically significant increase in leukaemia incidence was reported compared with the incidence for the general population (Ivanov et al, 1997a). However, the workers received frequent medical examinations, so that the accuracy and completeness of their leukaemia diagnoses are likely to differ from those for the general population. Indications that ascertainment biases may have produced this result come from a case–control study nested within the Chernobyl recovery operation worker cohort, which found no evidence of an increase in the incidence of leukaemia (Ivanov et al, 1997b). Again, it should be pointed out that ascertainment bias applies equally to both cohort and case–control studies. In the context of case–control studies, ascertainment bias can arise if the selection of cases or controls is influenced by exposure status. In such studies it is therefore important that there be comparable ascertainment for cases and controls, and in particular that ascertainment be as complete as possible for both groups. For example, when it is necessary to approach potential study subjects, or their relatives, for interviews, it is important that the refusal rate for both cases and controls be as low as possible.

It is sometimes necessary to approach cohort members, or their relatives, to recall exposures. This is very likely to be the situation when studies, in particular case–control studies, are organised retrospectively. 'Recall bias' arises when information, for example on exposure, is collected retrospectively, and patients, or their relatives, are subject to differential recall of this information, depending on their disease status. For this reason, much the strongest studies are those that rely upon independently maintained registers of exposure – for example, the registers of radiation dose that are maintained for regulatory purposes for many cohorts of nuclear workers (Muirhead et al, 1999, 2009).

Related to recall bias is 'investigation bias', which results if investigators scrutinise exposures more thoroughly for cases than for controls. Although register-based studies are not prone to recall or investigation bias, they are subject to errors due, for example, to inaccurate diagnostic information. To the extent that such studies should not be biased by knowledge of radiation exposures, it would be expected that random misclassification due to inaccurate diagnosis would not affect values of the ratio of the excess disease rate to the underlying disease rate in the absence of radiation exposure, that is to say the excess relative risk (ERR), although values of the excess disease rate itself, or the excess absolute risk (EAR), might be biased, either positively or negatively. In occupational cohort studies there is frequently a healthy worker effect, whereby workers, who are selected as being available for work in a particular cohort, will tend to be healthier than the general population, and so have lower mortality and incidence rates. As long as the appropriate (internal) analysis is conducted, this should not be a source of bias.

A 'confounding factor' is one that is correlated both with the disease under study and with an exposure of interest. Confounding factors can lead to bias. In many studies there is no reason to expect correlations between most factors and the radiation exposure, so that confounding ought not to be a problem. In studies of medical exposures, confounding may arise if the clinical indications that lead to the exposures are related to a subsequent diagnosis of the relevant disease; this is sometimes referred to as 'confounding by indication'. For example, in a study of patients administered iodine-131 for diagnostic

purposes, a slightly elevated risk of thyroid cancer was observed (Hall et al, 1996). However, this risk was not related to dose and was concentrated among patients referred because of a suspected thyroid cancer (Hall et al, 1996), indicating that the apparent elevated risk was probably due to the underlying condition. There are known to be correlations between smoking rate and the DS86 radiation dose among female survivors of the atomic bombing of Hiroshima, although there are no such correlations for the male survivors in this city, or for either males or females in Nagasaki (Preston, 1999). This may be connected with the (statistically non-significant) indications that the radiation-associated excess relative risk (ERR) of lung cancer increases with increasing age at exposure and attained age in this dataset (Little, 2002), findings at odds with the customary reduction of ERR with increasing values of these variables (UNSCEAR, 2006). However, it is clear from the analyses of Yamada et al (2004) and Shimizu et al (2010) that smoking does not confound the radiation dose–response for circulatory disease in this cohort.
Cigarette smoking is one of the most serious confounding factors that have to be dealt with in epidemiological studies. As shown in Table A1 (reproduced from Pierce et al, 2003), the ratio of the disease rate to the underlying disease rate in the absence of the relevant exposure (in this case to cigarette smoke), ie the relative risk (RR), of lung cancer associated with cigarette smoking – which for moderate to heavy smokers generally exceeds ten (Peto et al, 2000; Pierce et al, 2003) – is much greater than the RR associated with exposure to high doses of radiation (which rarely exceeds two). Therefore even slight confounding by factors related to cigarette smoking can seriously bias studies of lung cancer or other smoking-related cancers. When suitable information is available, confounding factors can be dealt with at the analysis stage, either by incorporation of such factors into the regression model, or by stratifying the data according to levels of the confounding factor.

TABLE A1 Lung cancer risks associated with cigarette smoking and radiation exposure for the survivors of the atomic bombings in Japan (Pierce et al, 2003)

Relative risk due to cigarette smoking [a]			Relative risk due to radiation exposure [b]
1–15 cigarettes per day	16–25 cigarettes per day	>25 cigarettes per day	1 Sv
4.9	8	13.3	2.2

Notes
a Relative risk adjusted for radiation exposure, age at exposure 30 years, attained age 60–70 years.
b Relative risk adjusted for smoking, attained age 60–70 years.

RCTs, cohort and case–control studies all use individual-related data, in particular data on individual exposures. By contrast, correlation studies are based on data averaged over groups, as noted above.
A particular form of this type of study is the 'geographical correlation study' (often referred to as an 'ecological study'), in which disease rates based on data aggregated over geographical areas are compared with aggregated data on levels of exposure – for example, to natural radiation or to man-made increases in environmental radiation levels. The possibilities for bias in such studies are well known. The principal cause of bias (sometimes termed 'ecological bias') is the failure to take account of correlations

within each area between multiple risk factors (eg radiation and smoking) (Greenland and Robins, 1994; Piantadosi, 1994). Examples of such studies include ones of leukaemia (Henshaw et al, 1990) and lung cancer (Cohen, 1995) in relation to environmental radon progeny exposure. The possibilities for bias in such studies are illustrated by a study of lung cancer in relation to environmental radon progeny exposure in Sweden, which when analysed as a case–control study yielded a positive slope for lung cancer risk versus radon progeny concentration, but when analysed as a correlation study, with grouped exposure estimates, yielded a negative slope (Lagarde and Pershagen, 1999).

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