I write these notes in the wake of a tsunami in Japan and popular anxieties on both sides of the Pacific about the release of radiation. Radiation, ionizing and non-ionizing, is a small part of the disease spectrum seen routinely by veterinary pathologists. Extensive epidemiologic and biologic evidence links ionizing-radiation exposure with cancer. Yet radiation injury is important in part to the popular fears it inspires. It is also important due to value and routine clinical use with conventional X-rays, computed tomography (CT) and magnetic resonance imaging (MRI), and in the treatment of cancer. Radiation from CT scans involve x100 – 500 more radiation than that in conventional X-rays, depending on which part of the body is imaged. The National Research Council (NRC) recently concluded that patients exposed to radiation in the range provided by one CT scan were at a low but increased risk of cancer. Currently, each year approximately 10% of the US population undergoes CT scans. Some 75 million scans are performed with use of CT growing by >10% annually. Radiation also offers a useful insight into DNA damage and its repair. There is in interesting medical history of the misuse of radiation for the treatment of various medical conditions, particularly in women and children (see slide #39). In veterinary medicine, radiation treatment is a common adjunct for cancer treatment. One of the most common endocrine tumors of cats affects the thyroid gland (adenomatous thyroid hyperplasia) and is successfully treated with radioactive iodine, since it accumulates selectively in the thyroid. One of the most effective treatments for aggressive equine sarcoids is the use of radioactive implants. In each case, while effective, such treatments carry risks both to the animals and to people handling or treating them. Anyone going into a medical career should be aware of the small, but real, risks associated with radiation.

Two forms of radiation (slides 1 – 6)

Radiation can be categorized as ionizing and non-ionizing. Non-ionizing radiation is important in animals as the basis for solar injury and, in consequence, many carcinomas of skin and conjunctiva. Medical radiation is response for <20% of our total exposure. Most irradiation comes from natural sources, particularly radon, and from food and cosmic sources. Medically, the most important form of non-ionizing irradiation is ultraviolet light. Conventionally this is categorized as A, B and C wavelengths, based on decreasing wavelength UV-A: 400–320 nm, UV-B: 320–280 nm, and UV-C: 280–100 nm. From a practical standpoint, UV-A is largely irrelevant. It can cause transient immunosuppression due to effects of irradiation on leukocytes trafficking thought the skin. UV-C rays are more damaging, but are not a major issue at present since atmospheric ozone deflects them from the earth and its inhabitants. That leaves UV-B. UV-B’s effects are due to reversible and irreversible injury to DNA. It is considered a complete carcinogen since no cofactor is required to induce cancer. The damage includes the formation of dimmers, base changes, cross-links, and single- and double strand breaks in DNA. All are reparable to varying degrees, but double strand breaks are the important as they are the most difficult to repair. These are the ones most likely to result in cell death, dysplasia/neoplasia, and senescent changes.

Acute UV solar radiation injury (slides 7 – 8)

Solar injury is common in domestic animals, particularly those housed outdoors at higher elevations. Sunburn is common in horses and dogs in Wyoming and may exacerbate two disease syndromes. One is the photo-aggravated autoimmune disease discoid lupus erythematosus (DLE; commonly called collie nose, although most breeds are affected). It is called DLE to distinguish it from the less common, but medically more serious, systemic lupus erythematosus, an autoimmune disease in which multiple host cellular components (primarily protein and host DNA) are targeted. Acute and subacute DLE results in apoptosis (“sunburn cells”) of keratinocytes in germinal layers of the skin. This leads to disorganization and edema. If sufficiently severe, there is vesicle (blister) formation due to dermoepidermal separation. Rupture of vesicles leads to the classical signs of severe acute sunburn with loss of epidermis. Solar irradiation causes chronic dermatisis and depigmentation that affects the nose, and less commonly skin of the eyes and mouth, when exposure is persistent for DLE-susceptible dogs. Other histological changes are fragmentation of elastin in upper dermis, and vascular leakage of capillaries. These are morphologically distinctive in both people and animals, so that when skin cancer occurs it is often possible to recognized evidence of solar dermatitis in the affected area. A second medically important form of UV irradiation is photosensitization. Photosensitization refers to increased susceptibility of skin to damage due to photodynamic agents, and is subcategorized into three types. One is due to ingestion of photodynamic plant toxins and drugs. This is the reason why, with drugs such as oxytetracycline, patients are recommended to avoid solar exposure. Specific plants – none of which grow in Wyoming, but do so elsewhere in the US – may trigger photosensitization. Another type is due to a variety of metabolic diseases

PATHOLOGY OF IONIZING AND NON-IONIZING RADIATION

PATB 4130/5130

D O’Toole
that result in the formation of endogenous photodynamic compounds. The most important group is due to breakdown products of heme (porphyrin and protoporphyrin; see Dr. Fox’s lecture) in the inherited diseases porphyria and protoporphyria. These are relatively rare in animals. Type 3 is the most important, at least in herbivores. The cause is liver damage, leading to hepatojenous phototoxic photosensitization. Since chronic liver damage is relatively common in horses, skin damage is often the first sign of hepatic failure. Dietary chlorophyll is converted to phyllerythrin by enteric bacteria. Phyllerythrin in absorbed, made harmless and soluble by conjugation in the liver, and excreted in bile. When there is insufficient reserve capacity in the liver, phyllerythrin acts as a photoaggravating agent. In all three types of photosensitization, unpigmented skin is most severely affected. The classical appearance (slide 8) is of ulcerated crusting skin lesions, typically affecting lightly haired, unpigmented skin such as nasal blazes, coronary bands, around the eyes, or on stockings.

**Chronic solar injury and skin cancer (slides 10 – 15)**

Chronic ultraviolet irradiation in people is associated with two forms of skin cancer: basal cell and squamous cell carcinoma (due to chronic low-level exposure), and malignant melanoma (generally considered to be due to periodic, intense exposure, particularly in childhood). It was estimated in 2000 that there were 2.8 million human cases worldwide of squamous cell carcinoma (SCC), 10 million cases of basal cell carcinoma (BCC) and 200,000 cases of malignant melanoma. BCC and SCC are among the most common skin cancers in people. They can be counted as “good” cancers, since they are readily identified, are slow to grow and metastasize, and readily removed surgically. They have their counterparts in cattle, horses and cats. Non-pigmented tissues in all four species are prone to squamous cell carcinomas. There is species variability in which part of the body is affected. In cattle they tend to involve conjunctiva and skin around the eye. This susceptibility to “cancer eye” is one reason why more heavily pigmented breeds have supplanted Hereford cattle, which have white-pigmented heads. By contrast, in white cats the affected tissue most often involves the nose (slide 13) and ears. For some reason basal cell carcinomas attributed to solar irradiation are rare. In addition, we see solar-induced tumors of the dermis in dogs, cats and horses that have not been associated with solar exposure in people. These dermal and conjunctival hemangioma and hemangiosarcomas (tumors derived from the lining of blood vessels) are relatively common in all three species. Their distinctive histological appearance and superficial location signals their basis in UV irradiation. A more serious neoplasm associated with UV irradiation is cutaneous malignant melanoma. As its name suggests, it originates in melanin-forming cells of the skin. One major function of melanocytes to protect keratinocytes from UV damage – this is the process that involves tanning. Each melanocyte protects an average of 30 – 40 basal keratinocytes. Aggregates of intracytoplasmic melanin granules form a cap over each keratinocyte nucleus. Malignant melanomas are important since they comprises less than 5% of malignancies in skin yet account for 60% skin tumors with a fatal outcome in people. A major triggering factor is thought to be intense intermittent solar exposure. Other factors, particularly genetic susceptibility, are involved. Malignant melanomas in people pass through a defined sequence of changes, summarized in slide 15. Each forms an aggregate of dysplastic and then neoplastic melanocytes in the epidermis. Initial growth is radial (horizontal) in skin, leading to a recognizable blemish with irregular margins and the common medical recommendation that all moles that change shape be medically examined. The medical mnemonic is ABCD, signifying asymmetry, border irregularity, color variegation, and a diameter of >6 mm. Once melanomas grow vertically into dermis, they become more serious. Depth of growth is correlated with an ability to undergo metastasis, with a heightened risk that vessels will invaded. One of the curiosities of UV-induced tumors in animals is that, so far as I know, they are not associated with malignant melanomas. There are several human genodermatoses where DNA repair is defective and as a result there is a heightened risk of skin cancer. These include xeroderma pigmentosum (XP) and Cockayne syndrome. XP is a rare autosomal recessive disease with a worldwide incidence of 4 in a million live births. Patients have a genetic inability to repair specific forms of DNA damage due to defects in nucleotide excision repair (NER) pathway. In healthy individuals this pathways removes helix-distorting DNA lesions from the genome. XP manifests as photosensitivity and an incidence of skin cancer some 1,000 times higher than average. It presents at 1-2 years with photosensitivity and sunburn. Later cutaneous manifestations are dry skin, numerous freckles, and telangiectasia (dilated blood vessels, in this case in skin). There is an increased incidence of skin cancer on sun exposed sites. Skin and conjunctival cancer develops at around 8 years of age in children who are not kept out of the sun. Genetically, XP is divided into subtypes (XPA-XPG) based on the nature of mutation. Cockayne syndrome is a similar but more serious disorder due to mutations affecting proteins which stabilize RNA polymerases. Both diseases may have a neurological component due to limited repair among cell populations in the central nervous system – this has nothing to do with UV irradiation.

**Irradiation and nuclear damage (slides 16 – 20)**

Given the importance of free radicals as a defense mechanism and the multiple ways in which DNA can be damaged by oxidation, alkylation, hydrolysis and deamination, it is not surprising that cells have considerable and redundant capacity for DNA repair. When radiation damage is severe, the cell’s response is straightforward: it undergoes apoptosis. Prompt cellular death ensures that
progeny mutant cells do not arise. With damage is sublethal, repair is highly efficient. This is the rationale for fractionating of radiation doses when used to treat neoplasia. Fractionation allows time for cellular repair or death to occur, before the dose is repeated. When one of two DNA strands are damaged, the undamaged strand can be used as a template to guide correction. Various excision repair mechanisms replace the damaged one with the appropriate nucleotide sequence. **Base excision repair (BER)** repairs damage to single bases, which is removed by a DNA glycosylase. The missing component is recognized by an endonuclease, is resynthesized by a DNA polymerase, and a DNA ligase performs the final nick-sealing step. Double strand breaks are more serious. Although repair mechanisms exit for such breaks, these are more likely to lead to genomic rearrangements. If the rate of DNA damage exceeds repair capacity, the accumulating errors can result in senescence, apoptosis, or dysplastic (pre-neoplastic) changes. In family lines where some component of DNA repair is limited, there is increased likelihood of neoplasia.

The severity of irradiation injury is determined by several factors. These include the type of ionizing radiation, dose and rate of delivery, whether irradiation is localized or involved the whole body, whether concurrent vascular injury occurred, and the proportion of actively dividing cells in the affected field. As a rule, rapidly dividing cells are more susceptible to ionizing irradiation than quiescent cell populations. It is for this reason that severe whole body irradiation is dominated by enteric clinical signs and susceptibility to infection: rapidly dividing cells of the gut and bone marrow are particularly affected. Patients who receive doses of radiation in the low to mid-lethal range have depressed bone-marrow function. If sufficiently severe, blood cell production is arrested and there is complete **pancytopenia** (reduced numbers of all cellular components in blood). Changes in the peripheral blood profile occur as soon as 24 hours post-irradiation. Lymphocytes are depressed most rapidly. The time of onset of the depression of cellular production in the marrow varies considerably, but the end result is uncontrolled hemorrhage, lowered resistance to infection, and anemia.

**Slides 21 – 29**

Radiation can be expressed in various and confusing ways. The SI units of grays (Gy) is a measurement of the amount of absorbed radiation (1 Gy = 1 joule of energy absorbed by 1 kilogram of matter or tissue). Typically, low doses are expressed as 1/1000 or 1/100 of a Gy (mGy and cGy, respectively). A CT scan of the human thorax typically involves exposure to 2 – 5 cGy. Biologically damaging doses are in the >2 – 5 Gy range. Cutaneous ulceration is induced by doses of >20 Gy (slide 26). Slide 27 summarizes the effects of irradiation on skin at different exposures. Hair follicles have a rapidly dividing population of cells in the hair bulb and can be transiently (>3 Gy) or permanently damaged (>7 Gy) by irradiation.

A serious aspect of ionizing radiation is the susceptibility of fetal tissues, hence the medical importance of avoiding exposure of pregnant animals and women. **In utero** effects depend on the time of gestation when irradiation occurs. Exposure to biologically significant concentrations of radiation is highest in early pregnancy, corresponding to fertilization, implantation and organogenesis. In human embryos and fetuses this corresponds to <25 weeks gestation. The incidence of fetal wastage consequential to significant concentrations of radiation is highest in early pregnancy, corresponding to fertilization, implantation and organogenesis. Inadvertent radiation at this age is unknown. Some women are unaware they are pregnant when exposed and the background rate of miscarriage is high (25 – 50% of all conceptions). Radiation injury in early gestation is assumed to be all-or-nothing; not many cells in an embryo need to be damaged before abortion occurs. Later in gestation the main risks are congenital malformations, mental retardation, reduced intrauterine growth, and childhood cancer. During the period of <25 weeks post conception, the central nervous system is particularly sensitive to radiation and radiomimetic effects. Fetal doses in excess of about 0.1 Gy may result in a decrease of IQ. Doses of 1 Gy result in a high probability of mental retardation particularly at 8-15 weeks post conception. The CNS is less sensitive to these effects at 16-25 weeks of gestational age, and largely resistant thereafter. The principal forms of radiation-associated childhood cancers are the leukemias (typically within 10 years of exposure; see below) and solid tumors, which tend to occur later in life and at lower rates.

**Acute radiation illness** (slides 30 – 35)

High doses of whole body irradiation (>50 Gy) result in an acute incapacitation syndrome and death. Lower doses of whole body irradiation primarily affect bone marrow and gastrointestinal system. Since the longevity of blood cell components varies between species, the time between exposure and clinical disease is species-dependent. But in each case the effects are the same: **anemia** (due to decreased red blood cell production), **increased bleeding** (due to reduced platelets) and heightened susceptibility to infection (due to **leukopenia**). Assessment of lymphocyte counts in individuals subjected to whole-body penetrating radiation within 48 hours of exposure is a reliable predictor of outcome. The normal lymphocyte count is in the 1,500 – 3,000 cells/mm² range. Cell counts of below 1,000 lymphocytes/ mm² confirm biologically significant exposure. Counts below 100 lymphocytes mm² carry a grave prognosis.
Chronic complications of irradiation (slides 36 – 40)

Chronic radiation damage is important since some local tissue injury is inevitable as part of radiation-based cancer treatment. The amount of injury is a function of which tissues were subjected to penetrating radiation. Glandular tissues are particularly susceptible due to constant turnover of cells. This is true for both exocrine tissues, such as salivary and lachrymal glands, and endocrine tissues. One complication of irradiation of facial tumors is atrophy of salivary glands resulting in permanent xerostomia. Endocrine deficiencies may occur after cranial irradiation due to adverse effects on the pituitary glands. These include growth primary hypothyroidism, gonadal failure, and obesity. A small proportion of adult female survivors of childhood cancer develop persistent ovarian failure. Offspring of women who had uterine irradiation as children are more likely to be born preterm or have low birth weight. Other long term effects are damage to white matter in the brain, which involves edema, coagulation necrosis, vascular alterations and demyelination. Some of these complications are potentially fatal, particularly when the target tumor is in a sensitive area such as the brain stem. Vessels are surprisingly sensitive to irradiation, although effects may not be recognized for years. These are due to damaged endothelium, with capillary rupture and thrombosis. Larger vessels exhibit proliferation of endothelium, leading necrosis of vessel walls and arteritis.

The most common major complication of whole body or intense localized ionizing radiation is leukemia. Therapy-related myelodysplastic syndrome and acute myeloid leukemia are direct consequences of mutational events, and tend to have characteristic mutations. Although the incidence is low, it is a major complication in that irradiation-associated leukemias tend to be difficult to treat successfully. Other, less common tumors are B-cell lymphomas and solid tumors of multiple organs (primarily lung, female breast, colon, and stomach). These complications have become important as the success rate for treating cancer has increased in children. Currently, as a result of better treatment, almost 80% of children and adolescents in the US receiving a diagnosis of cancer become long-term survivors. But Such survivors were eight times as likely as their siblings to have severe or life threatening chronic health conditions, including a second cancer (slide 41). Not all of this can be laid at the door of radiation since multiple treatments are used, but it is likely that irradiation is a major risk factor.

You will sometimes hear the time radiomimetic virus. The final two slides illustrate some examples. These are viruses that selectively target actively replicating cells. The common parvoviruses of dogs and cats, responsible for acute canine parvoviral enteritis and feline panleukopenia, target cells in the DNA synthesis stage of the cell cycle. As a result, some of their effects are remarkably similar to those of irradiation, in terms of enteric damage and leukopenia. Feline parvovirus can also cause radiation-mimicking effects in the central nervous system, such as cerebellar hypoplasia.

Donal O’Toole

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