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PET INVESTIGATION OF RADIATION INDUCED SEQUELAE

OF THE SPINAL CORD

Ph.D. Thesis

by

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1. Introduction

The discovery of X-rays by Roentgen in 1895 opened a whole new era in medical sciences (Roentgen 1896). Soon after the initial experiments aiming at diagnostic applications of radiation, physicians became aware of its unfavorable biologic side-effects. Therapeutic applications of gamma rays and later on other types of ionizing radiation were introduced into clinical practice in the early years of the 20th century. Following an initial boost it became quite clear that efficient applicability of radiation therapy depended mainly on selective sparing of normal tissues while delivering lethal dose to tumors. In order to improve radiation techniques and protocols many experiments were carried out on animals and clinical trials were also performed, and included critical analysis of tumor-response as well as radiation induced injuries in general. The pathologists described all the macro-, and microscopic changes brought about by ionizing radiation as well as the time course of specific cellular reactions. Despite experiments carried out on cell cultures that disclosed many of the biophysical and biochemical mechanisms that seemed to stand behind these processes, some obviously essential ingredients of the complex physiological response to radiation in any given tissue still remained in the shadow. The main drawback in the investigation of radiation-induced alterations at tissue level in humans was that techniques supposedly capable to disclose information about the morphological and functional details (i.e., blood supply, viability of cells, etc.) were profoundly dependent on histological samples obtained by biopsies or even more often gathered from autopsies. As a consequence, compiling relevant physiological and/or biochemical data on processes ongoing in various tissues during or shortly after irradiation was possible only on rare occasions. *In vivo* characterization of radiation induced pathological processes by functional imaging methods has only

recently become possible, most promisingly by positron emission tomography (PET). This highly sensitive technique utilizes short-lived, positron-emitting isotopes to label specific molecules that participate in physiological as well as in pathophysiological processes. Tomographic mapping of the distribution of intravenously (i.v.) injected tracers allows precise quantitation of an ever-increasing number of biochemical and/or physiological reactions *in vivo*. The relatively low radiation burden and the noninvasive nature of this particular functional imaging method make it an ideal tool for clinical research and human studies. Our intention was to apply this technique for a more detailed characterization of radiation myelopathy, gathering data heretofore unavailable.

Radiation myelopathy is a rare, but highly feared complication of radiotherapy. It typically occurs following the irradiation or reirradiation of malignant tumors located close to the spinal cord (SC) that are likely to respond to radiation (e.g. malignant lymphomas, seminomas, or certain types of thyroid, laryngeal and nasopharyngeal cancers). It may also be a consequence of application of state-of-the-art, high-dose radiotherapeutic protocols for otherwise incurable tumors (e.g., various forms of pulmonary, esophageal, head and neck cancer). Myelopathy may affect both the white and the gray matters. White matter involvement is more common, and it usually runs a chronic, progressive, irreversible and relentless clinical course, with only rare instances of even partial recovery from established damage (Jellinger and Sturm 1971; Kristensson et al. 1967; Palmer 1972; Reagan et al. 1968; Schultheiss et al. 1988 and 1995). Thorough research of the available relevant literature-databases on white matter damage has revealed only 7 well-documented published cases of remission (Dynes and Smedal 1960; Ésik et al. 1999; Glanzmann et al. 1976; Solheim

1971). In contrast, regeneration of sensory losses is reported with a relatively higher frequency (Schultheiss et al. 1995).

During the past decades, the unfavorable position and external circumstances for radiation therapists plus the often times inadequate instrumentation in Hungary inevitably caused radiation injury at a higher rate compared to the so-called "developed" countries. These cases serve as a pool of patients presenting the clinical sequelae of radiation induced spinal cord damage. Investigation of patients with manifest symptoms of spinal cord injury is a difficult task mostly because their general physical/medical condition. However, it is still feasible when post-therapeutic staging of the disease is required in order to pursue "optimal" – if possible – treatment of a particular disease. Subjects with complete irreversible functional loss fortunately are hard to find and those interested in this special field have to refer to cases, relevant to this type of injuries, reported in the literature (Suzuki et al. 2003; Tsuchiya et al. 2003; Hamandi et al. 2002; Yamaura et al. 2002; Pietronigro et al. 1985; Hecht et al. 1984; Kojima et al. 1979).

Although data about the pathomorphology of radiation related damage abound, previous PET studies (Regis et al. 1999; Inoue et al. 1995; Ishikawa et al. 1993; Miyatake et al. 1992, Ogawa et al. 1988; Di Chiro et al. 1988) have provided only limited information on the radiation-induced reactions of the central nervous system (CNS). Data on spinal cord damage are practically not available, since the reported investigations focus exclusively on the pathological changes of the brain, most probably because of the low spatial resolution of PET cameras. In these studies focal radiation necrosis of the brain was visible appearing as a region of decreased $[^{18}F]$-2-fluoro-2-deoxyglucose (FDG) uptake and reduced blood flow (BF). To our

knowledge, regeneration following radiation injuries in the SC has not as yet been studied.

Therefore we decided to investigate the various processes ongoing at tissue level by means of PET in patients who suffered from fully or partially reversible radiation injury of the spinal cord. We planned to attempt to relate regional blood flow, glucose metabolism and amino acid uptake of the myelon to clinical signs and symptoms and/or possibly to the results of histological analysis of tissue samples whenever available from these patients in order to get a deeper insight into the mechanisms underlying the restitution of function.

The results of PET imaging done on patients with or without clinical signs or symptoms of myelon damage together with those of the autopsy findings gathered from a similar population offer the chance to possibly describe the pertinent pathologic reactions in greater detail. This approach might also reveal mechanisms that as of now have yet not been perceived as factors important in the recovery process of function.

2. The normal and the radiation-injured spinal cord

a. *Anatomy and physiology of the spinal cord*

The spinal cord is located inside the vertebral canal, which is formed by the foramina of 7 cervical, 12 thoracic, 5 lumbar, and 5 sacral vertebrae, which together form the spine. It extends from the foramen magnum down to the level of the first and second lumbar vertebrae (at birth down to second and third lumbar vertebrae). The myelon is ensheated by the fibrous dura mater and a much more delicate pia-arachnoid layer. The spinal cord is composed of 31 segments: 8 cervical (C), 12 thoracic (T), 5 lumbar (L), 5 sacral (S), and 1 coccygeal (Co). The spinal nerves

comprise the sensory nerve roots, which enter the spinal cord at each level, and the motor roots, which emerge from the cord at each level (Fig. 1).
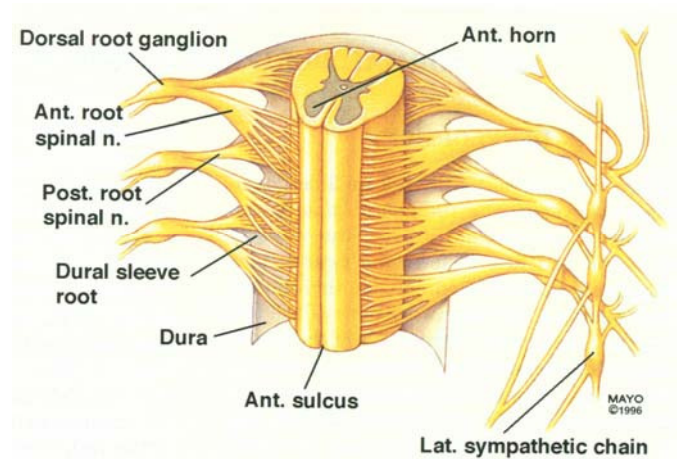
Fig. 1 *Schematic representation of the sensorimotor and autonomic nerves and their ganglia relative to the spinal cord and meninges.*
(Scheithauer WB et al. 1996)

The spinal nerves are named and numbered according to the site of their emergence from the vertebral canal. C1-7 nerves emerge above their respective vertebrae. C8 emerges between the seventh cervical and first thoracic vertebrae. The remaining nerves emerge below their respective vertebrae. The dorsal rami of C1-4 are located in the suboccipital region. C1 participates in the innervation of neck muscles, including the semispinalis capitis muscle. C2 carries sensation from the back of the head and scalp and provides motor innervation to several muscles in the neck. C3-C5 contribute to form the phrenic nerve and innervate the diaphragm. C5-T1 provide motor control for the upper extremities and related muscles. The thoracic cord has 12 segments and provides motor control to the thoracoabdominal musculature. The lumbar and sacral portions of the cord have 5 segments each. L2-S2 provide motor control to the lower extremities and related muscles. The conus medullaris is the cone-shaped termination of the caudal cord. The pia mater continues caudally as the filum terminale through the dural sac and attaches to the coccyx. The coccyx has only one spinal segment. The cauda equina is the collection of lumbar and sacral spinal nerve roots that travel caudally prior to exiting at their respective intervertebral foramina. The cord ends at the L1-L2 vertebral levels. Several macroscopic grooves

are discernible on the surface of the spinal cord. Most prominent is the anterior median fissure, which is occupied by the anterior spinal artery. The posterior median sulcus is less prominent. The anterior and posterior nerve rootlets emerge at the anterolateral and posterolateral sulci (Young and Young 1997).

Histologically the spinal cord is composed of a central area of gray matter in a shape of an H surrounded by columns of myelinated axons of the white matter. At the cellular level, in addition to neurons of the gray matter, both gray and white matters comprise astrocytes, oligodendrocytes, microglia (processes of astrocytes and to a lesser degree of microglia form the neuropil), that also harbors an intricate network of the microvasculature.

The blood supply of the cord is provided by the anterior spinal artery and two posterior spinal arteries (Fig. 2).
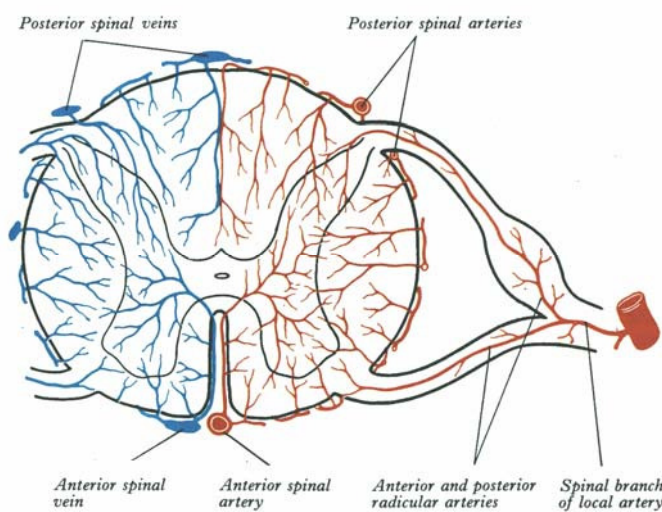
Fig. 2 *Diagram of the blood supply of the spinal cord. (Scheithauer WB et al. 1999)*

Proximally these arise from the vertebral arteries and at lower levels from the intercostal arteries. Smaller arteries form a dense anastomosis network along (outside) and also within the cord. This is described later in detail. Depending on the type and size of the vessel, their wall is comprised of myofibroblasts, smooth muscle cells plus elastic fibers. They are often surrounded by a rim of fibroblasts. Endothelial cells with

complex functions line the inner surface of the vessels. Ependymal cells constitute the wall of the central canal of the spinal cord (Schultheiss et al. 1995).

I. DEVELOPMENT

The development of the CNS is a very complex process regulated by an intricate, interactive system of molecular signalling network. Hereby we can only describe the most important steps in a simplified pattern.

On day 19 of the ontogenesis, indentations appear on the neural plate, demarcating the future forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon). On day 22, neurulation begins, with the neural plate folding into the neural tube, and with closure of the neural tube, bulges of the 3 primary vesicles appear. A ventricular layer of cells surrounding the neural canal proliferates to produce most of the cell types of the future CNS. The 1st wave of proliferation from this layer produces neuroblasts (future neurons) that migrate peripherally to form an outer mantle zone (future grey matter of spinal cord and brain stem). The nerve fibres from neuroblasts form a marginal zone, superficial to the mantle zone, which contains fibre tracts (future white matter of the spinal cord and brainstem). The 2nd wave of proliferation produces glioblasts that migrate peripherally and become astrocytes and oligodendrocytes. Finally, the ventricular layer differentiates into the ependyma lining the brain ventricles and central canal of the spinal cord. At the end of the 4th week, the mantle layer becomes organized into the paired basal (ventral) and alar (dorsal) columns of the ventral and dorsal quadrants of the spinal cord, respectively (Barr and Kiernan 1988).

Myelination of axons in the spinal cord begins in the corticofugal tracts during early infancy, from age of 6 to 8 weeks, then it progresses according to a specific pattern reaching a fully developed state around the end of the second year after birth.

II. WHITE MATTER – A BRIEF OVERVIEW OF ITS STRUCTURE

The white matter (WM) of the spinal cord is divided into 3 paired funiculi: posterior, lateral, and anterior. Each contains ascending and descending tracts comprised of myelinated and unmyelinated axons (Fig. 3).
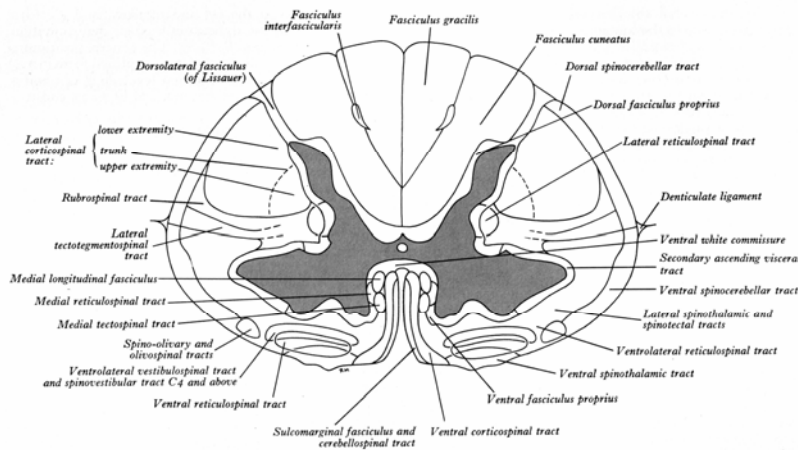
Fig. 3 *Diagram of a transaxial section of the spinal cord in the cervical segments indicating the main tracts.*
(Scheithauer WB et al. 1999)

Predominance of the myelinated fibers produces the white color. These axons may be involved in communication within a given individual segment of the spinal cord, (this group of axons generally remains within the gray matter) as well as between different segments of the spinal cord (this latter group of axons is located closest to the gray matter and is sometimes given its own name, i.e., *fasciculus proprius*) or to/from the brainstem and various forebrain structures (these ascending and descending long tracts that form various sensory and/or motor pathways constitute the bulk of the white matter). The anterior white commissure is a band of transversely oriented nerve fibers, which are crossing from one side of the spinal cord to the other. Almost all crossings in the spinal cord, whether sensory or motor, take place in the anterior white commissure. The amount of white matter progressively increases from S to C levels (Parent and Carpenter 1996).

Myelin (80% lipid and 20% proteins) is formed in the CNS by the compaction of the plasma membrane of the oligodendrocyte process that enwrap a part of the length

of the axon. Thus the myelin sheath around an axon is derived from oligodendroglial cells. The myelin sheath is interrupted by constrictions at varying intervals on axons. These areas are revealed by electron microscopy as regions where the myelin sheath is deficient, i.e., gaps known as nodes of Ranvier. The axons are continuous across the nodes. Myelin ensheathment of an axon is associated with faster conduction of impulse that requires less energy compared to the unmyelinated fibers (Vabnick and Shrager 1998).

III.   CONCISE DESCRIPTION OF GRAY MATTER

The grey matter can be divided into 10 laminae (Rexed's laminae or layers) or into 4 parts: anterior or ventral horn (i.e., motor neurons; laminae VIII, IX, and part of VII), posterior or dorsal horn (i.e., sensory part; laminae I-VI), intermediate zones (i.e., associate neurons; lamina VII), and lateral horns (i.e., part of the intermediate zone, present in the thoracic and lumbar segments, where sympathetic neurons are located). The spinal gray matter neurons are also arranged into columns or nuclei. The substantia gelatinosa and proper sensory nucleus extend throughout the whole spinal cord and receive pain impulses. Other nuclei such as the nucleus of Clarke are present only in certain segments. The anterior arms, or horns, of the gray matter contain large motor nerve ganglion cells (neurons), while the posterior horns contain smaller, sensory neurons. Anterior (ventral) horn enlargements are most prominent at cervical (C5 toT1) and lumbosacral (L3 to S1) levels. These enlargements are formed by the large number of motoneurons required to innervate the muscles of the upper and lower extremities, respectively. The intermediolateral cell column is present only at T1 to L2. This column (or nucleus) is formed by cell bodies of preganglionic sympathetic neurons (Young and Young 1997).

IV. THE SALIENT FEATURES OF BLOOD SUPPLY OF THE CORD

Arterial supply

The spinal cord is supplied by descending branches of the vertebral arteries (that eventually merge to form the anterior spinal artery) and multiple radicular arteries derived from segmental vessels. The anterior spinal artery enters the anterior median fissure of the spinal cord and supplies the anterior two thirds of the cord. It also supplies midline rami to the lower medulla. Like the basilar artery, it has smaller penetrating and circumferential branches.

The two posterior spinal arteries supply the ipsilateral posterior one sixth of the cord (or combined, the posterior one third). They receive varied contributions from the posterior radicular arteries and form two longitudinal plexiform channels near the dorsal root entry zone. Radicular arteries are derived from segmental vessels (e.g., ascending cervical, deep cervical, intercostal, lumbar, and sacral arteries) that pass the intervertebral foramina and give rise to anterior and posterior radicular arteries. Segmental radicular arteries supply blood to the roots, and segmental radiculospinal arteries supply the roots as well as the cord. Usually a few large segmental radiculospinal arteries are noted, including the artery of Adamkiewicz (or artery of the lumbar enlargement), which is larger than the others; it usually originates between T9 and T12 (in 75% of cases) and supplies the lower one third of the cord. Where two anterior radicular arteries reach the same level of the spinal cord, a diamond-shaped arterial configuration develops. The distance between radicular arteries is greatest in the thoracic spinal segments, thus occlusion of one thoracic radicular artery may seriously compromise circulation. Therefore, the upper thoracic (T1-4) and L1 segments are particularly vulnerable to vascular insults (Barr and Kiernan 1988).

Venous drainage

Veins draining the spinal cord have a distribution similar to that of the arteries. Anterior longitudinal trunks consist of an anteromedian and an anterolateral vein. Sulcal veins drain the anteromedian portions of the spinal cord. Blood from the anterolateral regions of the spinal cord drains into the anterolateral veins. Posterior longitudinal venous trunks drain the posterior funiculi. The internal vertebral venous plexi (i.e., epidural venous plexi) are located between the dura mater and the vertebral periosteum and consist of two or more anterior and posterior longitudinal venous channels that are interconnected at many levels from the clivus to the sacral region. At each intervertebral space are connections with thoracic, abdominal, and intercostal veins and external vertebral venous plexi. These spinal veins have no valves, and blood passes directly into the systemic venous system. The continuity of this venous plexus with the prostatic plexus (via the so-called Batson-veins) is an important path along which prostatic cancers metastasize.

Blood – spinal-cord barrier

The spinal cord (tissue compartment) is separated from the intravascular compartment by the blood – spinal-cord barrier, which protects the neurons and the other cellular elements by restricting the passage of potentially harmful substances through the walls of the blood vessels. This barrier is composed of highly specialized endothelial cells that line blood vessels within the CNS that regulate the transport of molecules from the blood into the cellular environment (inter- and intracellular compartments). This is brought about by structural and functional properties of the endothelium. Tight junctions between the endothelial cells block the movement of water soluble molecules. At these tight junctions the adjacent layers of neighbouring endothelial cell membranes fuse creating a pentalaminar site. The foot processes of

astrocytes provide a further protecting, regulating layer abutting on the continuous basal lamina of the vessels' wall. Another part of the barrier is the negatively charged glycocalyx on the inside surface of the endothelial cells. This serves to repel plasma proteins that also bear a negative charge. The integrity of the blood-spinal-cord barrier is very important since it provides not only a stable micro-environment that is essential for normal neural function, but also (via specialized transport systems) supports the transport of essential substances (e.g., glucose, amino acids, etc.) for the neural tissue (Misra et al. 2003).

V.   RENEWAL OF THE SPINAL CORD ELEMENTS, CELL KINETICS

The estimated number of ganglion cells (neurons) in the CNS is on the order of 10 billion. Although the most recent observations show that some neurons might be replaced by new cells even in adults and even become capable of forming functional connections, it is fair to say at this juncture that neuronal regeneration is very limited and the whole issue is under intensive research. Mature neurons do not divide, but their function and viability may be seriously affected by ionizing radiation. Astrocytes form an extensive framework of glial fibers in both the white and gray matter. Astrocytic foot processes are attached to all sides of the walls of the microvasculature and are part of the blood-brain barrier. The astrocytes normally divide slowly if at all, but divide rapidly in response to injury and may then produce gliosis, or a "cerebral scar". Oligodendrocytes normally are also slowly dividing cells, but they may respond to injury by more rapid mitosis. These cells produce myelin and maintain its integrity. The number of intrinsic microglial cells seems to be constant normally, however they rapidly respond to CNS injury; however their rapid increase in number, results almost exclusively from an influx of blood monocytes (macrophages) derived from bone marrow stem cells. Perivascular cells (of which there are 5 different types) also do

participate in reactive processes. Endothelial cells (ECs) are assumed to have a slow turnover rate but respond promptly to injury from ionizing radiation or other harmful noxae. Because of the complex functions of endothelium, the injury may lead to equally complex, often poorly understood reactions (necrosis, apoptosis, anoikis etc.) Smooth muscle cells, fibroblasts, and myofibroblasts of the vascular walls, with an extremely slow cell turnover rate, are still susceptible to radiation-induced injury. Over time, the irradiated intimal and adventitial tissues of the blood vessels often produce profound abnormalities (fibrinoid necrosis, thrombosis, etc.) that may assume a dominant role in the development of the so-called delayed radiation injuries of the CNS (Fajardo et al. 2001).

Due to differences between the kinetic features of the cells involved and variability of their susceptibility or sensitivity towards injurious noxae (i.e., *selective vulnerability*), radiation-induced injury may develop acutely (in days or weeks) or may be delayed for months or years of apparent quiescence before becoming an identifiable cause of clinical signs or symptoms (Schultheiss 1995).

VI.   ELECTROPHYSIOLOGY OF NERVE CONDUCTION

Information between different foci in the nervous system is transmitted (exchanged) and this process is based on various mechanisms, one of which is the generation of action potentials (AP), also called nerve impulses. These phenomena are defined as "rapid, transient, self-propagating reversals in membrane potential" (McClintic 1985). All living cell membranes have electrical potential across them, that is usually referred to as resting potential. In its resting state, the membrane is characterized as "polarized". The value of the resting potential varies between different cells, from 5 to 100 mV. The inside of the cell is electrically negative relative to its environment. The establishment of this resting potential is closely related to the active

transport of Na^+ and K^+, since the membrane is more permeable to K^+ than to Na^+. The active transport is fueled by energy provided by the Na^+/K^+ ATPase.

Neuronal and other excitatory membranes have a membrane potential value, termed the threshold, that has to be reached, or in other words to which the membrane must be depolarized to initiate an AP. Once initiated, the AP is conducted in decrementless fashion along a normal axon, meaning that there is no decrease in the intensity of the impulse. If a stimulus can cause depolarization of the neuron's membrane, the response is all or none. Axons possess a refractory period, the time during which they are in the depolarized state. The length of the refractory period is about 1 msec. The axon is ready to conduct another impulse very quickly. Many nerve fibers show accommodation, evidenced by a rise in the threshold of the fiber. The more slowly the stimulus depolarizes the fiber, the greater the intensity of the stimulus must be to initiate an AP. This phenomenon may enable the fiber not to respond to stimuli that persist once any given area or locus of the CNS has detected or "recorded" any kind of stimuli. Under proper circumstances, two or more stimuli that are individually only subthreshold can add together to initiate an AP. This phenomenon is called summation. Temporal summation occurs when two stimuli are applied in close succession to a single fiber. There is a local partial depolarization from one stimulus that is furthered by the second stimulus. Spatial summation occurs when two subthreshold stimuli are applied simultaneously but at different points on a neuron. They "merge" to cause depolarization and development of an AP.

In nerve cell axons that do not have insulating myelin sheaths, local current flow develops from positive to negative areas at the point of local suprathreshold depolarization. The current flow is of sufficient strength to depolarize the next segment, which develops an AP, that causes depolarization, and hence current flow that induces

depolarization of the next segment, and the process continues in this pattern. In summary, depolarization and repolarization occurs in a step-wise manner as Na^+ and K^+ channels open and close in adjacent parts of membrane. This is called continuous impulse conduction. Axons are normally stimulated at one end, so the impulses are conducted in one direction due to the so-called absolute refractory period, that is the time-frame during which the membrane is unable to respond to stimuli of any strength.

In myelinated axons the current flow occurs only at such foci where there are gaps in the sheath - that is, at nodes of Ranvier. This results in the AP being developed at the nodes that usually lie 2 to 3 mm apart (this distance is quite variable in the different parts of the CNS and peripheral nervous system /PNS/). The impulse will then cover a given distance in jumps (from node to node, bypassing internodes) rather than steps, speeding up its rate of passage along the axon. This type of conduction is called saltatory conduction. This type of conduction is much faster and also saves ATP, because Na^+/K^+ pumps are active only at the nodes.

b. Pathology, pathophysiology and clinical features of radiation-induced spinal cord injury

The spinal cord may get irradiated during radiotherapy of tumors. The most common primary SC tumors within the spinal cord and its coverings are astrocytomas, ependymomas, meningiomas and nerve sheath tumors within the spinal canal (Bowers 2003). Any of these may get treated by radiation therapy (N.B. meningiomas are rather radio-resistant!). Locally invasive or metastatic cancers of the spinal cord, vertebral bodies (osteo-, chondro-sarcomas or plasmocytomas) are more likely to require irradiation. Perhaps most frequently, the spinal cord receives radiation while it is included in the radiation fields applied for therapy of neoplasms of the head and neck, larynx, lungs, and mediastinum, as well as various malignant lymphomas and Hodgkin's

disease. The lumbar cord is prone to radiation injury during therapy of cancers of the pancreas, kidney, the biliary tract, metastatic prostate cancer. Also, para-aortic lymph nodes harboring metastases (urinary tract and testicles having the primary tumor) or lymphomas and various sorts of retroperitoneal tumors are often irradiated.

No single pathognomonic characteristic has been identified for the lesions of radiation injury of the CNS. Selective damage to the gray matter would most likely eliminate radiation as the causative agent of injury. Demyelization and necrosis (malacia) are common forms of a host of diseases affecting the white matter. Demyelization and malacia are consistently the dominant morphological features of clinical and experimentally induced radiation myelopathy (Schultheiss 1992).

Although damage and loss of nerve fibers are inevitable components of the lesions in symptomatic cases, the responses of the vasculature and glial cells are highly variable and, as a result, add controversy to the interpretation of the pathogenesis. The lack of invariant morphological changes induced by the irradiation of the CNS is a reflection of the similarities between radiation responses of the CNS and CNS's reactions to other causes of damage. These responses extend beyond the cells that are killed by the initial insult. Some reactions to injury are brought about as an attempt to repair or removal of the damaged tissue, while several cellular/biochemical processes that are activated by various noxae may actually contribute to the damage.

Early injury may appear 2 weeks after completion of irradiation. More often, the evidence of injury is recognized only several months later. Early irradiation injury of the human spinal cord has not often been studied by pathologists (Rubin and Casarerr 1968). Focal areas of demyelination may be found in the white matter columns of the cord. These (at least at first glance) are rather similar to the plaques of acute multiple sclerosis (MS). More thorough work up, however, discloses that radiation damages or

destroys axons as well as the myelin sheaths. Later, spongiform change is seen in the white matter. Eventually, (4-5 months after irradiation), small vessels (mainly arterioles or small arteries) show thickening of their wall frequently accompanied by fibrinoid necrosis. Endothelial cell proliferation, fibrin thrombi, and perivascular edema with a fibrin (or even albumin) exudate are present, and teleangiectasias are common (almost pathognomonic) findings. Veins as well as arteries are damaged; indeed, there are indications, that veins may be more severely injured. If the medulla and pons are included in the radiation fields, they show similar lesions.

Delayed radiation injury of spinal cord may develop anywhere between 6 months to many years after irradiation. White matter necroses frequently mimicking tumor-like masses of the cord every so often are present, and spongiform degeneration plus vascular lesions develop. White matter coagulative necroses are often small and may be multiple. Large, poorly defined masses that cause enlargement of the cord may also be found, so much so that they may be misleading and are often prone to raise the possible misdiagnosis of a neoplasm. Older necroses may contract ("shrink"), reducing the size of the cord. The resulting gross appearance may be that of a ribbon of gliotic, fibrotic organized granulation tissue (fibrous "scar") with or without multiple cysts. Foci of calcification are not rare in the area of older necroses. Differentiation of large coagulative necroses (especially those with hemorrhages) from ischemic, or for that matter hemorrhagic infarcts may be difficult, therefore the relevant clinical data and adequate neuroradiological evaluation are mandatory to be at hand for the pathologist (e.g., anamnestic information, laboratory data, MRI). Foamy macrophages and nodules of activated microglial cells as well as reactive astrocytes in and around older necroses are often numerous. The astrocytic proliferation at the margins, even to the point of replacing smaller necroses, may be found. Wallerian degeneration (axonal bulbs and

ovoid bodies) occurs in nerve tracts damaged by the necroses. Axonal reaction indicates neuronal damage and may be present quite distantly from the necrotic areas. Autopsies of irradiated patients who developed progressive motor weakness, various types of paralysis, or even hemiplegia show demyelination of the motor and sensory tracts. The causative irradiation may often have been administered many years earlier (Schultheiss 1995).

As with postradiation injuries of the brain, the pathogenesis of damage to the spinal cord is multifactorial. Reduced blood flow due to vascular (capillary) damage (vide supra) is of profound importance. Small arterial, arteriolar and small vein (venules) injuries further compromise blood flow. Direct radiation injury of parenchymal cells, with loss or reduction of the function of oligodendroglia, is a major player in the demyelination process. Damage to astrocytes, and disruption of their anatomic and functional relationship to blood vessels (i.e., blood-tissue barrier) and hence increased capillary permeability lead to edema and fibrin, albumin exudation. Injury (i.e., occlusion) of the vasculature is a major factor in the formation of necroses. Necroses may arise as liquefactive foci that eventually transform to fibrin-rich coagulative destruction of the tissue. As with the brain, the role of injurious ECs products (leukotriens, cytotoxic metabolites, etc.) and cytokines released from injured cells, as well as the accumulation of free oxygen radicals also play a significant role, however, the exact mechanisms (the interaction of all these factors) remain to be elucidated in the pathogenesis of postradiation myelopathy.

I.   WHITE MATTER INJURY

Consistent features of radiation lesions in the spinal cord are singular or combined white matter lesions that are listed in an estimated order of increasing severity in Table 1. This order that is based on severity of the lesions does not necessarily reflect

or imply a strict, much less a probable temporal sequence of development of these lesions. Furthermore, the morphological severity does not reflect morbidity *per se*. Morbidity is more related to the extent, size, and location of the lesions than to specific morphology. Schultheiss et al. have categorized the various radiation myelopathy lesions observed in autopsy material. Type 1 lesions involve only the white matter or have only minor vascular changes assumed to be insufficient to produce symptoms. Type 2 lesions are predominantly vascular in nature and the various forms of white matter damage (if any) are considered to be secondary to the vascular damage. Type 3 lesions are complex and have characteristics of primary damage to both the white matter and the vasculature.

Table 1. White matter lesions in order of increasing severity[1]

1. Demyelization: isolated nerve fibers
2. Demyelization: groups of nerve fibers, tracts (spongiosis)
3. "Inactive" malacia
    a. spongiosis and spheroids
    b. scar
4. "Active" malacia
    a. coagulative malacia
    b. liquefactive malacia
        i. amorphous (complete necrosis)
        ii. foam cell fields
        iii. cystic
5. Hemorrhagic malacia

There is not a single target cell type and a single pathway of radiation-induced damage of the CNS. Oligodendrocytes and endothelial cells are usually regarded as most vulnerable, henceforth being the potential target cells. The general view is that these cells have both independent and overlapping roles in the pathogenesis. Their relative and interrelated contribution is bound to be influenced by radiation dose and most likely by other factors. The emphasis on oligodendrocyte injury rests on the premise that reproductive death and failure of replenishment of these cells account for

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[1] Modified after *Schultheiss ET et al. 1995.*

white matter destruction. However, it is unlikely that death of the slowly proliferating oligodendrocytes alone would result in the eventual development of confluent lesions of demyelination and outright malacia. The accent on oligodendrocyte death has been modified by considering that the loss of these cells may be furthered by failure of their replenishment due to elimination of glial progenitor cells.

It is well recognized that most often vascular injury is the primary cause of necrosis. However, it is inconceivable that white matter necrosis would result from vascular injury, particularly when thorough search fails to prove morphological vascular abnormalities somewhere in the irradiated field. Furthermore, no cogent explanation has heretofore been offered for the mechanism by which malacia would result from mitotic death of oligodendroglia. In many other demyelination diseases (e.g. MS), denuded axons are frequently observed without axonal loss that is so characteristic of radiation injury. Furthermore, it is not clear why slowly proliferating oligodendrocytes, with as yet unidentified, remaining stem cells in adult animals, should undergo a sudden and dramatic reduction in number at highly variable times following irradiation. In addition, one would expect that mitotic death of oligodendrocytes from radiation would result in diffuse demyelination, however, this is not obligatory, since focal necroses are often observed adjacent to apparently normal white matter.

II.   GREY MATTER INJURY

Besides white matter reactions, grey matter sequelae (neuronal degeneration, pyknosis, chromatolysis, and dispersion of the Nissl substance) may also occur in the anterior and posterior horns of the spinal cord, but the grey matter *per se* is less radiosensitive than the white matter.

The damage as such caused by radiation to the grey matter is the result of the combination of its direct lethal effect on the ganglion cells and glial elements plus the

secondary cellular deterioration due to vascular injuries. The occurrence of pure gray matter damage in an extent sufficient to elicit clinical symptoms without significant lesions in the white matter is extremely rare and usually affects the motor neurons in the anterior horn.

Microglia and astrocytes commonly exhibit profound alterations in the irradiated spinal cord. As noted in Table 2, the pattern of increased numbers and greater prominence of these cells may be diffuse or focally concentrated to areas of parenchymal destruction. Astrocytes and microglia probably play an active role in radiation injury, e.g., release of cyto- or histotoxic substances are quite likely after these cells suffer injury. Meanwhile their participation in reparation and phagocytosis (removal of debris) is beyond debate. It is also worth to keep in mind that these cells, once activated and/or injured may be major players in antigen presentation and thus initiation of further injury by immunological mechanisms.

Table 2. Summary of the glial reactions to irradiation[2]

1. Microglia/macrophages
    a. morphology
        i. rod-shaped
        ii. foam cells
        iii. multinucleated "giant" cells
    b. patterns
        i. diffuse
        ii. focal
        iii. perivascular – this may occur in
            combination with either (i) or (ii)

2. Astrocytes
    a. morphology
        i. inconspicuous, mild alterations
        ii. gemistocytic transformation
        iii. fibrillary predominance with
         conspicuous "star" shape and long processes
    b. patterns
        i. diffuse
        ii. focal

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[2] Modified after *Schultheiss ET et al. 1995.*

III.   VASCULAR INJURY

The vascular lesions encountered in the irradiated spinal cord are listed in Table 3. These vasculopathies are also found in other conditions, thus they cannot be considered to be full-proof indication of radiation damage or absolutely pathognomonic, however, in cases when radiation has been clearly documented and they are present with other alterations as outlined above, they are highly indicative of changes caused by radiation. Like other tissues, the irradiated spinal cord may contain vascular lesions without parenchymal changes. The list of known vasculopathies is presented in Table 3 to emphasize that parenchymal lesions may occur without altered vascular morphology. However, vascular lesions, especially in combination with demyelination and malacia, comprise a picture that can be considered to be highly characteristic of radiation myelopathy both in humans as well as in nonhuman primates.

Table 3. Vasculopathies with or without endothelial alterations of hyperchromasia, hypertrophy, anisokaryocytosis, and hyperplasia[3]

1. None
2. Irregularly increased vascularity
3. Telangiectasis
4. Hyaline degeneration (change) and thickening of vascular wall
5. Perivascular edema and fibrin exudation
6. Perivascular fibrosis and inflammation (mostly lymphocytic)
7. Bona fide vasculitis
8. Fibrinoid necrosis of the entire wall
9. Thrombosis (often microthrombi)
10. Hemorrhage (frequently perivascular and patchy)

Sometimes it is difficult to make clear distinction between precapillary arterioles and postcapillary venules in histological sections of the CNS. Even though vasculopathies associated with radiation injury apparently occur in both arteries and veins, certain features seem to suggest preferential venous damage. Cases without morphological indication of neuronal death in radiation myelopathy argue against

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[3] Modified after Schultheiss, E. T. *et al.* 1995.

arterial impairment. It was noted that monkeys with radiation myelopathy consistently had lesions in the lateral corticospinal motor tract. This is an area that is commonly, although not solely affected by venous lesions.

Slowly evolving and delayed changes in the structure and permeability of the microvasculature had historically been emphasized as primary causes of radiation injury of the CNS (Hornsey et al. 1981; Hubbard and Hopewell 1979; Myers et al. 1986). An essential role for vascular injury cannot be denied in some cases of radiation myelopathy when there are obvious lesions of the vasculature, e.g., necrosis, thrombosis, and manifestations of altered permeability indicated by edema, fibrin deposition, and hemorrhage. However, an indispensable role for vascular injury might be challenged on the basis of reported cases with malacia with morphologically normal or minimally altered vessels (Morgan 1985). Although individual cases can either support or contradict the contribution of vascular changes to the pathogenesis. Indeed, experimental evidence supports the likelihood of injury to both glial cells and blood vessels (Cicciarello et al. 1996; Husain and Garcia 1976). What determines their relative contribution to the destruction of the neuropil remains to be clarified.

Evidence supporting microvascular injury as a critical event in radiation myelopathy comes from recent investigations (Goodman et al. 1989) with boron neutron capture therapy (BNCT). The central feature of BNCT is the selective delivery of a boron compound to the tissue with consequent release of alpha particles following capture of epithermal neutrons by the boron. The alpha particle [^{10}B(n,α)^{7}Li] has a short range of 5-9 μm and its damage is limited to the cells in the immediate vicinity. Brain tumors were chosen for initial studies because the boron is confined to the normal microcirculation due to the tightness of endothelial cells, i.e., the blood-brain barrier (BBB) that virtually excludes most compounds, unlike the leaky brain-tumor barrier

(BTB). Two lines of evidence suggest that BNCT induced microvascular injury leads to focal necrosis. Gavin et al. (1994) noted lethal brain necroses in normal dogs secondary to multifocal hemorrhagic infarctions. More compelling is the work of Morris et al. (1994), who produced radiation myelopathy with BNCT, delivering the ^{10}B in sodium borocaptate ($Na_2B_{12}H_{11}SH$ or BSH) molecules. With calculated doses of 5.7 Gy to the CNS parenchyma and a calculated 33 Gy dose affecting the blood proper (since the nuclear reaction also takes place here) that enters the spinal cord vasculature, it is evident that endothelial cells can also be target cells.

IV. MIXED DAMAGES

Type 3 lesions are characterized by simultaneous primary multifocal deterioration in the cellular components of the white matter and of the vasculature, the latter being more severe than only hyaline thickening and intimal proliferation. Inflammatory reaction represented by mononuclear cells (lymphocytes, mainly T cells, macrophages and microglia) was observed with greater frequency in Type 3 lesions. The average latent periods in patients with inflammatory reactions were shorter than in those without inflammation (Schultheiss et al. 1988).

V. CLINICAL SYNDROMES

Because the various signs and symptoms of radiation myelopathy can occur in many combinations and at different rates of progression, it is not possible to make the diagnosis of radiation myelopathy based solely on clinical signs and symptoms. The initial signs are subtle and may not be noticed by the patient. These may include: sensory deficits (either unilateral or bilateral) that often manifest themselves as diminished temperature sensation, leg weakness, clumsiness, and diminished proprioception. Lhermitte's[*] sign may precede permanent radiation myelopathy.

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[*] For detailed description see page 53.

Objective signs and symptoms include changes in gait (often foot drop), spasticity, weakness, hemiparesis, Brown-Sequard syndrome, and possibly incontinence. Hyperreflexia and Babinski signs are often found on neurological examination. Pain may accompany these symptoms. In many cases, the patient may have been asymptomatic until some trauma initiated a progressive neurological deficit. No combination of signs or symptoms distinguishes radiation myelopathy from myelopathies of many other etiologies. The severity of the symptoms is usually progressive, but may stabilize at any level. As time elapses, the symptoms are often attributable to damage affecting increasingly higher anatomical levels.

The clinical picture of the rare isolated gray matter injury involves radiogenic lower motor neuron disease (LMND), i.e. flaccid paresis due to the damage to the motor neurons situated in the anterior horn of the spinal cord. However, it may accompany a predominant radiogenic white matter injury (demyelination and/or ischemic vascular injury) with spastic motor, sensory and vegetative losses. In these cases, the grey matter injuries are manifested primarily in malfunction(s) of the upper limbs. Radiogenic LMND, however, is mainly reported as the only sign of the radiation injury and in these situations it involves basically the lower extremities of young, testicular cancer patients exhibiting a chronic, progressive and irreversible clinical course.

To make a diagnosis of radiation myelopathy, three criteria must be met (obviously it is prerequisite that the patient had had radiation therapy). First, other etiologies must be eliminated. By far the most common cause for myelopathy in a cancer patient is tumor progression, metastases or paraneoplastic syndromes. In addition, one should consider trauma or central neurological disease. Second, the presentation of symptoms should be consistent with radiation myelopathy. This would eliminate cases with upper extremity symptoms in the absence of lower extremity

symptoms, or those with pain as the only symptom. Third, the radiation dose and time to expression of injury must be consistent with a spinal cord radiation injury. A latency of less than 6 months is rare, and patients who received cord doses of less than 50 Gy are not generally at risk for radiation myelopathy. If the patient had received radiation to the spinal cord and the symptoms are consistent with radiation myelopathy but the dose or latency is not, one should consider whether any predisposing factors for radiation injury have been present. Such factors may include chemotherapy, previous CNS diseases, including vascular pathologies, and a number of other factors such as viral infections or trauma, developmental abnormalities, etc. Even without these predisposing factors, radiation myelopathy may remain one of the most significant candidates for causative diagnosis, particularly if the patient's history reveals prior radiation. The ambiguous "idiopathic myelopathy" is better be discarded, and continuous pursue to find the exact cause of the disease must be mantained (Abadir 1980; Zulch and Oeser 1974).

Typically, one obtains the following results from a diagnostic workup for radiation myelopathy. The myelogram is either negative or shows slight widening of the cord. Complete myelographic blocks have been reported in severe cases. Results of a thorough analysis of cerebrospinal fluid (CSF) obtained by a spinal tap are generally normal. Slightly elevated total protein-, elevated myelin basic protein concentrations, and lymphocytosis may be encountered (Paulson and Quenemoen 1984). Computerized tomography (CT) scans are rarely abnormal. Magnetic resonance imaging (MRI) may show cord swelling, decreased intensity on T1-weighted and increased intensity on T2-weighted images are indicative of edema (Wang et al. 1992). FLAIR MRI may be helpful. Decreased spinal nerve conduction velocities are often seen (Dorfman et al. 1982; Snooks and Swash 1985). Proper and adequate neurological workup frequently

reveals the symptoms described above. Special emphasis is warranted when Babinski signs are observed and the differential diagnosis of Guillain-Barre syndrome should seriously be considered. However, a slowly progressing radiation myelopathy is difficult to distinguish from any types of myelopathies, characterized by demyelinization.

c. Experimental data on the renewal of the spinal cord elements following irradiation

The details that characterize human myelopathy have been learned largely through clinical observations and less often through pathologic studies. Therefore extensive experimental investigations have been necessary to be conducted to learn the details of the morphologic lesions of the irradiated spinal cord. It has been difficult to correlate features of radiation injuries induced in small animals with short spinal cords and brief life spans with characteristics of radiation injuries induced in the human spinal cord. For these reasons, studies on dogs, pigs, and nonhuman primates are preferred, since they are more likely to provide relevant information.

Since clinical data on radiation myelopathy are scarce (Ruckdeschel et al. 1979), it is close to impossible to obtain precise knowledge on the specific importance of various biological factors, while trying to determine the radiation tolerance of the human spinal cord. Consequently, clinicians are, in general, extremely cautious in modifying treatment parameters and protocols. Hence animal studies are essential to develop basic concepts and provide data that would be applicable to clinical practice. Therefore a brief review of these data is in order.

I.   MODELS DESCRIBING RADIATION RESPONSE

For the proper interpretation of experimental data given in fraction sizes larger than 2 Gy, both the variable exponent nominal standard dose (NSD[*]) isoeffect formula and the linear-quadratic (LQ[#]) model appear to be appropriate. For daily fractions less than 2 Gy, the LQ model seems to provide less reliable data than the variable exponent NSD (Wong et al. 1992). Incomplete repair of radiation damage between multiple daily fractions require that both formulae be modified. Using the LQ model, it appears relatively safe to use a value of α/β of 2 to 2.5 Gy to estimate the biologically equivalent dose (BED[♦]) for various fraction sizes when treatment is delivered in daily fractions larger than 2 Gy. However, valid statistical analyses of clinical dose response data are unavailable, and clinical judgment must prevail over predictions from mathematical models. The overall time factor is negligible in the experimental setting when the duration of treatment does not exceed 2 months.

II.   STUDIES ASSESSING REPAIR KINETICS AND THE EFFECTS OF REPEATED TREATMENT

From the point of view of radiation response the cellular components of the SC are slowly proliferating, rendering the SC to be a lateresponding normal tissue. Similarly to what occurs in most tissues, the spinal cord injury is reduced if fraction doses are reduced while keeping total radiation dose constant. Studies in monkeys and rabbits have shown that the repair of radiation-induced injury of the spinal cord is slower than in most other normal tissues (Thames et al. 1982; van der Kogel 1991). The spinal cord tolerance dose was reduced 10% to 15% with accelerated hyperfractionation

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[*] NSD = $D / N^x \cdot T^y$, where D is the total dose, N equals the number of fractions and T is the overall time; $\underline{x}$ and $\underline{y}$ are exponents for different tissues.
[#] According to the LQ model $E = \alpha d + \beta d^2$, where E is the effect of radiation, $\underline{d}$ is the dose-per-fraction; α and β are factors describing the nonreparable and reparable components of the damage, respectively.
[♦] BED = nd $\{1 + d/\alpha/\beta\}$, where $\underline{n}$ denotes the number of fractions, $\underline{d}$ the dose per fraction, and α/β relates to a dose bringing about identical amounts of radiation-induced reparable and nonreparable damage (Fowler 1989).

(applying two daily sessions of only 1.2 Gy each with 6 to 8 hours intervals between them) relative to that of conventional day-long intervals. Studies on rats revealed that increasing doses were necessary to produce the same spinal cord injury by a second irradiation treatment conducted after increased length of time interval between the primary and repeated treatment.

The contribution of the time factor in determining the radiation tolerance of spinal cord, assessed with schedules that delivered two fractions with increasing intervals between irradiations, was found to be insignificant for a duration of 6-8 weeks for the cervical and high thoracic region of the cord. In the lumbar cord, effective recovery was shown to start between the 2nd and 4th weeks, but the degree of recovery was much less complete than in tissues with rapid cell turnover such as epithelia (van der Kogel 1979; White and Hornsey 1980). Some dependence of these results on the rat strain involved was observed.

Since multistep radiation-treatment is a critical issue in human radiotherapy, the rate of repair allowing a consequent retreatment has been studied in other animals. Neonatal guinea pigs irradiated with a single 10 Gy brain and spinal cord exposure are as tolerant of retreatment 1 year later as animals without prior irradiation (Knowles 1983). The spinal cord tolerance of monkeys 2 years after a 45 Gy (in 20 fractions) radiation treatment was identical to that of the control animals.

Although studies in rodents provided convincing evidence that the spinal cord has a large capacity to recover from occult radiation injury, it is not possible to extrapolate animal data to the clinic because of the tremendous differences between the life span of rats and humans. Rodent models allow assessment of intervals of at most 1 year, whereas retreatment in humans is usually considered after a number of years. Therefore, experiments using rhesus monkeys were performed to evaluate the tolerance

of the spinal cord to reirradiation (Ang et al. 1993). The cervical spinal cord of rhesus monkeys received ^{60}Co irradiation in 2.2 Gy fractions. The initial course of treatment consisted of total doses of 70.4 Gy, 77 Gy, or 83.6 Gy delivered for 15, 6, and 8 animals, respectively. The number of responders in these groups was three, three, and seven, respectively (Schultheiss et al. 1990). The 12 asymptomatic animals that received 70.4 Gy were reirradiated 2 years later to cumulative doses of 83.6, 92.4, or 101.2 Gy (four animals per group). Only two animals (receiving 83.6 Gy, and 92.4 Gy, respectively) developed paresis. Another group of animals received 44 Gy and 2 years later were reirradiated to cumulative doses of 83.6, 92.4, 101.2, or 110 Gy (four animals per group). Again only 2 of 16 (receiving 101.2 Gy, and 110 Gy, respectively) developed myelopathy. Asymptomatic animals were observed for at least 2 years following the last irradiation.

Several conclusions can be drawn from this study. First, there is no appreciable difference in the latent period for developing myelopathy between the single course and retreatment groups. This contradicts retrospective clinical observations (Schultheiss et al. 1984). Second, the majority of occult injuries induced by the initial radiation course are recovered within 2 years. For example, the ED_{50} value of the single course irradiation was 76.1 ± 1.9 Gy (standard error of the mean: SEM), whereas that of retreatment after 44 Gy was greater than 110 Gy. This means that the majority of occult injuries induced by the initial 44 Gy are recovered in 2 years. Third, the slope of the reirradiation dose-response curve is shallower than that of the single course treatment, which suggests variability in the rate of recovery among animals.

The observation that the majority of occult injuries induced by a dose of 44 Gy in 20 fractions (similar to the dose that the spinal cord receives in most patients with head and neck cancers undergoing definitive radiotherapy) are recovered within 2 years

might have an impact on treatment recommendations for previously irradiated patients. Further studies are under way to corroborate the currently available results by increasing the number of animals per dose group, assessing the dependence of the extent of recovery on the size of the initial dose, and determining the kinetics of long-term recovery. In addition, further attempts are required to develop methods to measure the degree of residual injury that may aid in determining the tolerance of the spinal cord of individual animals to retreatment.

As the survival of patients with certain tumors increases, radiation oncologists are more frequently faced with the problem of treatment of late recurrences or second tumors situated within or close to a previously treated site. For example, approximately 25% of head and neck cancer patients cured from their first tumors go on to develop second primaries. At present, radiation oncologists are reluctant to treat such patients because of the belief that one cannot retreat previously irradiated tissues, particularly the spinal cord. Knowledge of the kinetics of decay of occult injury from the previous treatment (i.e., reparation and/or regeneration) is required to rectify this misconception and to develop a rational treatment paradigm. However, data on tolerance of previously irradiated cord to retreatment are scarce. Limited clinical experience on retreatment of recurrent brain tumors and nasopharyngeal cancers suggests the existence of long-term recovery in the CNS (Dritschilo et al. 1981; McNeese and Fletcher 1981). A recent study with fractionated irradiation confirmed this finding (Mason et al. 1993).

III. CHARACTERISTIC FEATURES OF DOSE-FRACTIONATION

Extensive experimental data on the relationship between dose-fractionation and tolerance for the spinal cord have been reported. van der Kogel and others have demonstrated that the isoeffective dose increases exponentially with increasing number of fractions (van der Kogel and Barendsen 1974). The log-log plot of the

interrelationship of these entities allowed to fit the data by a regression line with a slope (characterizing the exponent of the number of fraction in the above function) of 0.37 - 0.44. The slope of the regression line reflects the capacity of cellular repair processes, steeper slopes being associated with higher capacity of repair. These findings are consistent with clinical reports (Phillips and Buschke 1969).

Spinal cord investigations provided additional evidence for the fact, that late-responding, generally slowly proliferating, (radiation) dose-limiting normal tissues are more sensitive to changes in fraction dose than are either normal tissues proliferating more rapidly or the majority of tumors (Thames et al. 1982). Consequently, late-responding tissues are preferentially spared when the dose per fraction is reduced. This observation has led to the reintroduction of hyperfractionation to exploit the differential effects of fractionation on various classes of tissues in an attempt to improve treatment outcome.

IV.   ALTERNATIVE FRACTIONATION SCHEDULES

In order to improve treatment outcome by altering radiotherapy schedules, it is essential to know the specifics about cellular repair kinetics in various tissues. Two prototype schedules, hyperfractionation (use of larger numbers of smaller fractions) and accelerated fractionation (use of shorter overall times than the conventional 5 to 7 weeks), are being tested in clinical trials. Although the rationales for these regimens are different, both types of fractionation schedules necessitate delivery of two or more fractions on each treatment day. To accommodate such a requirement in clinical departments, the interval between irradiations has been reduced from the conventional 24 h to 3 - 6 h, depending on the type of treatment schedule used. This logistic constraint may jeopardize the potential benefit of altered fractionation schedules if it

compromises the normal tissue tolerance to a larger extent than it affects chances of tumor control, because of incomplete repair between irradiations.

The impact of repair kinetics on the results of altered fractionation has been studied in rodents (Ang et al. 1984 and 1985; Thames et al. 1988; van der Schueren et al. 1988). Irradiations were given in 2 Gy fractions. Three conclusions resulted from these studies. First, the data can be interpreted in terms of a two-component biexponential repair model with $T_{1/2}$ of 0.7 h (95% confidence limits: 0.2-1.3 h) for the faster component and a $T_{1/2}$ of 3.8 h (2.6-4.9 h) for the slower component. Although the biexponential model describes the data satisfactorily, it does not provide information about the underlying mechanisms of repair. However, the proportion of repair through the slower component was estimated to be 62% (37%-86%). Second, shortening the interfraction interval to less than 1 day results in a significant decrease in spinal cord tolerance as a consequence of the long halftime of the slower component of repair. The isoeffective doses decreased by 16% and 13% when interfraction interval was reduced from 24 h to 6 h and 8 h, respectively. This observation should be taken into account in designing new fractionation schedules. Finally, the kinetics of repair characterized in these studies provides an explanation for previous findings showing that hyperfractionation did not result in as much increase in ED_{50} (the dose at which 50% of the subjects show effect) values as had been predicted by the LQ model for dose response (Ang et al. 1985; van der Schueren et al. 1988). When the effect of incomplete repair was included, the LQ model was found to describe isoeffective doses correctly for fraction sizes down to 1.2 Gy. However, these rodent data also indicate that when the CNS is the dose-limiting organ, the improvement of the therapeutic ratio (defined as the ratio of tumor killing to normal tissue damage) by hyperfractionation would be very small if two fractions per day were given in less than 8 h intervals.

V.   PRIMATE STUDIES

Studies on rhesus monkeys have revealed the similarity of spinal cord radiation response between this animal model and humans in terms of dose-incidence relationship and median latent period of manifestation of myelopathy (Schultheiss et al. 1992). It was concluded that this primate model is appropriate for the preclinical testing of new therapy strategies and investigating applied radiobiological questions, such as volume effect and reirradiation tolerance. Rodent models are not appropriate for these kinds of tests because of size constrain and limited life span. Subsequently, the compounding effect of incomplete repair in determining the tolerance of spinal cord to hyperfractionated regimen was investigated using this model by determining the extent of sparing when daily fractions of 2.2 Gy was changed to two fractions of 1.2 Gy per day with 8 h interval. The results indicate that the LQ model without incomplete repair corrections does not describe the data well and the dose-response for hyperfractionated treatment is shallower than for conventionally fractionated treatment.

Summarizing the essential findings obtained by the use of the rodent and primate models for clinical radiotherapy one can conclude that the interfraction repair in spinal cord is slower than previously anticipated. Therefore, there is little advantage in using interfraction intervals of less than 6 h. Shortening the interval between irradiations from 24 h to 6 to 8 h reduces the spinal cord tolerance by 10 to 15%. This should be taken into account whenever the CNS tolerance is dose limiting.

VI.   VOLUME EFFECT

It has been generally accepted that the dose to the spinal cord should be reduced when the volume, that is, the number of cord segments, to be irradiated is large, as it is in mantle field irradiation. This tradition has evolved based on the belief that the radiation volume is a strong determinant of the cord tolerance. No clinical data actually

support the existence of a volume effect in the spinal cord. Therefore, experimental studies are needed to determine the relative importance of this factor. Rat models are useful in investigating the mechanisms of volume effects but are less suited for obtaining clinically applicable data because the length of the cord in this species is only approximately 2 cm. Recently, larger animal models (primate, dog, and pig) have been used to evaluate the relationship between radiation volume and cord tolerance. The results from studies on primates and pigs (van den Aardweg et al. 1994) indicate that this relationship is consistent with the probability model predicting that the volume effect will be diminished in organs with steep dose-response functions (Schultheiss et al. 1983). Using the parameters of the model for the rhesus monkeys, one finds that to maintain a 1 % complication rate while doubling the length of irradiated spinal cord, the dose would be decreased by less than 4%. This means that in treatment settings for radiotherapy of head and neck or lung cancers, where the probability of myelopathy is less than 1%, changes in treatment volume has an imperceptibly small impact on the cord tolerance. It is recommended not to compromise tumor dose under the assumption that a longer length of irradiated cord diminishes spinal cord tolerance.

3. Patients and methods

a. *Patients and radiation protocols*

We have carried out PET studies on a total of 5 patients suffering from different forms of radiation induced damage of the spinal cord in order to complete the information having been obtained by conventional imaging and non-imaging diagnostic methods. One patient represents a case with complete recovery of the spinal cord following a subthreshold dose of irradiation (Patient 1). Two nasopharyngeal cancer patients were investigated (Ésik et al. 2003) due to permanent Lhermitte's sign after

radiotherapy (Patient 2 and 3). We have reported on PET findings in a patient with partially reversible radiation myelopathy (Patient 4), which were later correlated with autopsy results after her demise (Lengyel et al. 2003). A patient with radiogenic lower motor neuron disease was also investigated (Ésik et al. 2002) by PET (Patient 5).

Patient 1 (a 47-year-old woman) presented with a solitary, right submandibular lymph node measuring 1 cm in its largest diameter had been first observed 3 years prior its surgical removal in 1996. She was a smoker whose medical history included an earlier 10-year period of alcohol abuse, but she had later been abstinent for more than 10 years. The pathological diagnosis was Hodgkin's disease. Cervical ultrasound (US) and CT, chest CT, and abdominal US and CT suggested stage IA disease, and she received one cycle of chemotherapy. A follow-up second medical opinion excluded Hodgkin's disease, in view of the atypical location and the long-standing single lymph node involvement; this latter opinion was further supported by the subsequent negative gallium scan and negative whole-body FDG PET study. The pathological revision yielded the diagnosis of reactive lymphoid hyperplasia.

The follow-up had been particularly thorough because of the preceding diagnostic controversy. In February 1998, after a 22-month symptom-free period, [^{11}C]methionine PET had revealed intense tracer accumulation by two lymph nodes measuring about 1 cm in the right parajugular lymphatic region and another tracer-accumulating lesion measuring 1.5 cm in the right hypopharyngeal region. Laryngoscopic evaluation of the hypopharyngeal lesion was consistent with a primary carcinoma ($T_1N_{2b}M_0$) of the right aryepiglottic fold. The PET-demarcated boundaries of the viable neoplastic tissue allowed for a successful partial hypopharyngeal resection and radical right cervical lymph node dissection. The surgical intervention was followed by external irradiation with 6 MV photons and 8 MeV electrons between June 8 and

July 10, 1998. The intention was to treat the tumor bed and the bilateral parajugular lymph nodes with a maximum midplane dose of 50 Gy, using daily right and left portals with an angled-down technique (2 Gy/fraction/day, 5 times a week). When a midplane dose of 40 Gy was reached, the field size was shrunk and the spinal cord was no longer exposed directly to the irradiation. The posterior part of the field was boosted by 10 Gy electrons. Thus, the radiotherapy that involved the cervical spinal cord comprised a total dose of 40 Gy, with a calculated BED ($\alpha/\beta = 2$ Gy) of 80 Gy_2.

Patient 2 (a 41-year-old uranium miner for the previous 18 years) was diagnosed with a nasopharyngeal cancer (T_2 N_{2b} M_0) in 1976. He then received telecobalt irradiation with a curative intention, the relevant doses of which are given in Table 4. Besides the total dose and daily fraction dose, the biologically effective dose is indicated, presuming white matter injury in the cervical spinal cord.

A 43-year-old female (Patient 3) had been diagnosed as having stage I extranodal (nasopharyngeal) Hodgkin's disease in 1995 (the histopathology was reviewed and reconfirmed in 2001). She received telecobalt irradiation, with the relevant doses displayed in Table 4.

Table 4. The relevant characteristics of radiotherapy of the investigated patients with Lhermitte's sign

	Patient 2	Patient 3
Cervical spinal cord total / fraction dose (Gy)	48.3 / 2.3	46.2 / 2-2.2
BED for the cervical spinal cord (Gy_2)	103.8	94.8
Tumor total / fraction dose (Gy)	60 / 2	41.8 / 1.8-2
C 2-3 vertebral total / fraction dose (Gy)	69 / 2.3	41.8 / 1.8-2

A 36-year-old woman (Patient 4) had been operated on for multifocal papillary thyroid cancer (bilateral subtotal thyroidectomy with lymph node excision; stage pT_2 pN_{1a} M_0). She received postoperative telecobalt irradiation during 1990 (Ésik et al. 1999). The intention was to treat the tumor bed and the bilateral cervical lymph nodes from the mastoid process to the jugulum (with the exception of the accessory chains) with a maximum midplane dose of 50 Gy, using right and left portals daily with an angled down technique. Review of the radiation treatment chart revealed that the radiotherapy was conducted in three series involving 31 fractions during 81 days. Due to miscalculation of the depth of the midplane, and an erroneous daily bilateral application of the dose calculated for a daily unilateral treatment the patient received 3.4 Gy daily fractions during the total of 18 days of the two initial series. During the 13 days of the third series, the daily fraction amounted to 1.7 Gy. In the first two series (with a total duration of 45 days, including the break between them), the field covered the cervical part and the two rostral thoracic segments of the spinal cord. The length of the irradiated part of the spinal cord was 16 cm (the distal segments of which were probably exposed to a lower dose as a consequence of the larger body diameter). During the third series, the field size was shrunk and the spinal cord was no longer directly exposed. Thus, the radiotherapy involved a maximum spinal cord dose of 61 Gy. The overdosage explain the clinical symptoms indicated in the chart: the therapy was interrupted twice because of severe acute radiation-induced mucositis and epidermatitis. Moreover, the patient had moderate subcutaneous fibrosis and teleangiectasia within the irradiated region, reflecting the late tissue damage due to the high dose delivered. During year 2000, the otherwise apparently healthy patient had been complaining for months of short episodes of dyspnoe, but investigations by experts in relevant specialties (oto-rhino-laryngology, neurology and radiation oncology) had not revealed

any definite explanatory reason. Later during the same year, she had suddenly lost consciousness resulting from a central respiratory arrest that could not be clinically explained or accounted for. The subsequent 2 weeks of assisted ventilation did not bring about any improvements, while she acquired acute meningitis that eventually led to her death.

The history of Patient 5 (31-year-old male) involves previous, successfully treated pleural tuberculosis (medication with streptomycin, isoniazid and pyrazinamide between 1974 and 1976) and a motorcycle accident (1977, concussion). He had been hemicastrated for left-sided seminoma of the most common hystological type in stage $pT_2 N_0 M_0$ in 1985. Postoperative lymphography revealed no lymph node metastases. During the first postoperative month, the patient had received 1 cycle of chemotherapy (Vincristine 4 mg, Adriamycin 80 mg, Cyclophosphamide 2400 mg, Cis-platinum 80 mg and Bleomycin 60 mg, subdivided into 4 identical weekly doses). Adjuvant telecobalt irradiation was initiated on the penultimate day of chemotherapy, with the intention of treating the abdominal paraaortic and left parailiac lymphatic regions with a maximum midplane dose of 44 Gy, using anterior and posterior portals daily (2 Gy/day, 5 times a week), resulting in a BED of 88 Gy_2.

The control case (for spinal cord PET investigations) was a 49-year-old female with early hypopharyngeal cancer. PET imaging was performed before the initiation of her radiotherapy. No other noteworthy illness features in her history.

b. *Diagnostic imaging methods*

Radiation myelopathy is generally considered to have no pathognonic MRI signs. It is safe to state that the signs accompanying this disease and detected by MRI, such as edema in the acute disease, gadolinium enhancement in the subacute period and atrophy in the chronic phase, are aspecific (Bowen et al. 1996; de Carolis et al. 1986;

Feistner et al. 1989; Lamy et al. 1991; Tallaksen et al. 1997). For this reason and in order to gain a deeper insight into the processes underlying the investigated case histories, we applied functional imaging as well.

I. MRI

MRI investigations were performed on a 1.5-T system (Magnetom Vision Plus, Siemens, Erlangen, Germany) with a phased-array spine coil. FLAIR and T1-weighted turbo spin-echo sagittal sequences were obtained followed by T2-weighted turbo spin-echo axial scans.

II. PET

PET investigations were performed with a GE 4096 Plus scanner (General Electric, Uppsala, Sweden), providing 15 two-dimensional sections over an axial field of view of 103 mm. The optimum scanner resolution was 5.5 mm in plane and 6 mm in the axial direction. FDG and [11C]methionine studies were made after a 4-h fasting. The doses applied were 5.55 MBq/kg FDG, 9.25 MBq/kg [11C]methionine and approximately 2 GBq/scan [15O]butanol, and the examinations started from the base of the skull after a lag period of 40, 20 or 0 (immediately) min. following the administration of the tracers. Data for FDG and methionine imaging were acquired 10 min per bed position and 3 min for butanol studies (two [15O]butanol injections were performed in each subject). Attenuation correction was based on 15-min transmission scans performed in each bed position. Iterative reconstructions were made with a maximum likelihood algorithm optimized with respect to the computation time, developed in the Research Center, Jülich, Germany (Lipinski et al. 1997). The reconstructed image resolution was about 6 mm. For numerical comparisons, standardized uptake values (SUVs) were determined in the axial plane of the PET images by placing regions of interest (ROIs) covering the spinal cord between the

appropriate vertebral bodies to characterize both irradiated and nonirradiated segments (Ramos et al. 2001). The average activity per milliliter in any given ROI was found by applying a calibration factor obtained by scanning a uniform cylinder containing a known activity concentration. The uptake in this region was then corrected for the injected activity and the weight of the patient.

The PET examinations had previously been approved by the Ethical Committee of the University of Debrecen and the patients had given their informed consent.

c. *Electrophysiological studies*

Electrodiagnostic testing included electroneuronographic (ENG) and electromyographic (EMG) investigations. ENG was performed on the peroneal and posterior tibial nerve bilaterally to look for diminished peripheral motor or sensory conduction velocity by measuring the mean velocity of conduction and comparing it to the normal value. The amplitude of the action potentials were also recorded and compared to standards. The sensory and motor conduction velocities were also measured on the right and left median and ulnar nerve.

EMG was performed on the right and left quadriceps muscles, the anterior tibial muscle and the triceps muscle on the calf to look for signs of peripheral neurogenic lesion and fibrillation.

d. *Radiobiological and pathological methods*

The radiobiological investigations were approved by the Hungarian Health Care Scientific Council and the patients again gave their informed consent. Four sets of different studies were performed to check whether any kind of an individual high radiosensitivity might have played a role in the development of radiation-induced spinal cord injuries. First, a primary fibroblast cell culture had been established from a skin biopsy (Freshney 1987) and then, in a clonogenic assay (Elkind and Whitmore 1967;

Zhou et al. 1998), fibroblasts were irradiated with different doses of γ-radiation, and the survival rates were compared with the clonogenic survival of primary fibroblast cultures isolated from foreskin samples from 6 healthy children. Second, in a single cell electrophoresis (comet) assay (Klaude et al. 1996; Singh et al. 1988), whole blood had been irradiated with 2 Gy of γ-radiation followed by a comet analysis either directly after the irradiation to measure the initial DNA damage, or 4 hours later to allow time for DNA repair and determination of the residual damage. Data evaluation was carried out using the Komet Analysis System software package (Kinetic Imaging Ltd, UK). The initial and the residual DNA damage in the lymphocytes of the patients were compared with the results obtained on 43 samples collected from healthy individuals. Third, the spontaneous and the 2 Gy γ-irradiation-induced micronucleus frequencies were measured in a micronucleus assay (Guo et al. 1998) and compared with controls (Köteles et al. 1993; Kormos and Köteles 1988). Finally, the presence of chromosome aberrations was checked after in vitro irradiation of peripheral blood samples (Bauchinger et al. 1989) and compared with controls.

Histopathological analysis included Woelcke myelin staining for the demonstration of demyelination as well as for the presence of thin remyelination at the edges of the demyelinated areas. Axonal loss was evaluated by Bielschowski's method. We searched for the signs of motor neuron degeneration using Nissl's staining. Hematoxylin-eosin (HE), Periodic acid-Schiff (PAS), trichrom, van Gieson and Ag stained sections were analyzed for signs of vascular-wall alterations.

4. Results and discussion

a. PET studies on the recovery of the spinal cord following a subthreshold dose of irradiation

In 1996 a very low, background FDG accumulation was observed in the cervical spinal cord of Patient 1, during the investigations which led to the diagnosis of Hodgkin's disease (not shown), and also in 1998 (mean SUV: 0.84), before the initiation of radiotherapy (Fig. 4).
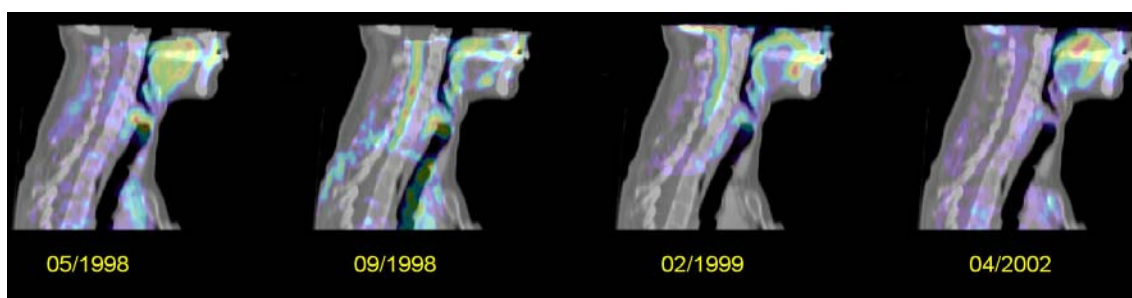

Fig. 4 *FDG PET examinations of the head and neck region reveal the normal, rather low /baseline/ (05/1998) uptake of the spinal cord, which was increased within the irradiated cervical spinal cord segments 3 months (09/1998) and 8 months (02/1999) following irradiation, and then decreased by 46 months (04/2002). The consecutive images (median sagittal sections of the fused PET-MR image data) display SUVs on an identical color scale.*

An increased FDG uptake was measured 3 months after radiotherapy (09/1998, mean SUV: 1.69), which was followed by a decline, as measured 8 months later (02/1999, mean SUV: 1.21). The FDG accumulation in the irradiated segments of the spinal cord decayed to a level very close to the initial value 46 months after the irradiation had been completed (04/2002, mean SUV: 1.11).

The simultaneous [^{15}O]butanol uptake results demonstrated a pattern of perfusion changes similar to those observed in the FDG studies (Fig. 5).
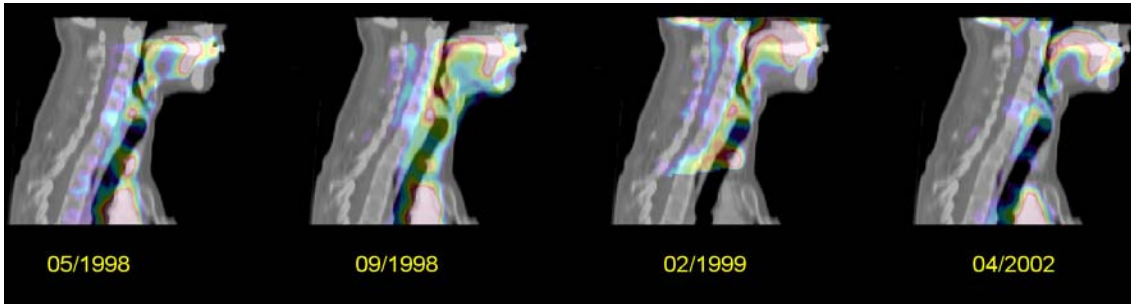
Fig. 5 *[^{15}O]butanol PET examinations of the head and neck region reveal an increased uptake within the irradiated cervical spinal cord segment, indicating increased tissue perfusion, paralelling the results of the FDG examinations.*

The patient had an extremely low [^{11}C]methionine uptake (Fig. 6) within the nonirradiated and irradiated segments of the spinal cord and this has not changed during the clinical course (the mean SUVs were between 0.35 and 0.25). The bone marrow uptake disappeared from the irradiated vertebral bodies C2-D3 (C1 has practically no body).
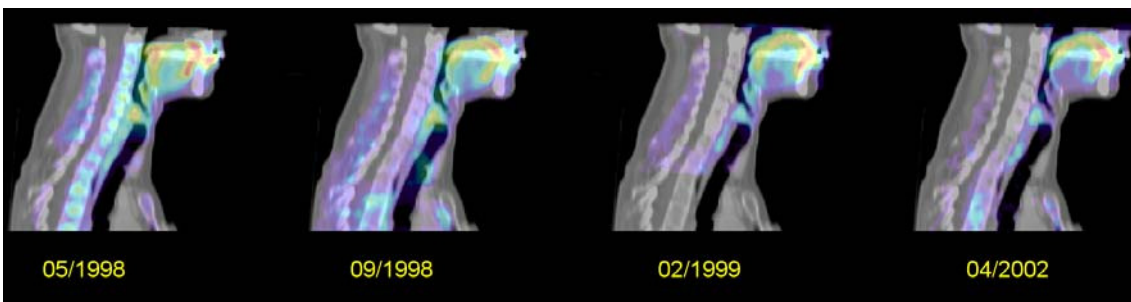

Fig. 6 *[^{11}C]methionine PET examinations of the head and neck region reveal only the normal, near background uptake within the irradiated and nonirradiated spinal cord segments. There is no physiological tracer uptake in the bone marrow of vertebral bodies C2-7 and D1-3. The consecutive images display SUVs on an identical color scale.*

In the fourth year of the clinical course (April, 2002), radiobiological investigations were performed. The fibroblasts of the patient displayed a slightly increased radiation sensitivity relative to those of the healthy controls (Fig. 7a).
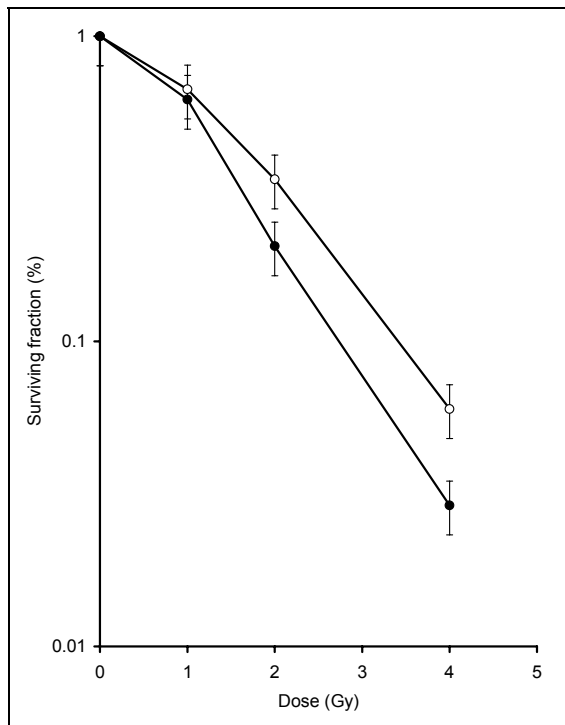
Fig. 7a *Survival curves with standard deviation for primary fibroblasts cells. Filled circles: fibroblasts from the present patient; open circles: average survival of fibroblasts from 6 healthy children.*

In the comet assay, no essential difference was found between the DNA repair capacity of the patient's lymphocytes and those of the controls.

The few studies that have been published on PET of the spinal cord revealed a very low physiological FDG uptake (Di Chiro et al. 1983; Kamoto et al. 1998; Meltzer et al. 1998; Wilmshurst et al. 2000; Woesler et al. 1997) and also a rather low [^{11}C]methionine uptake (Ésik et al. 1999 and 2002; Higano et al. 1990; Sasajima et al. 1996; Wilmshurst et al. 2000). A close direct relationship was described between regional blood flow and glucose metabolism of the spinal cord (Ésik et al. 1999 and 2002), which is very similar to the coupling of these parameters observed in brain studies (Roland 1993).

After radiotherapy of the spinal cord with a BED of 80 Gy$_2$, MRI and conduction velocity investigations provided normal results. At the same time, the irradiated segment of the spinal cord exhibited increased FDG and [^{15}O]butanol uptakes, which continually decreased during the 4-year clinical follow-up. The coextensive, analogous pattern of the variation in accumulation of these two tracers is in complete

agreement with previous findings (Ésik et al. 1999 and 2002). The spinal cord displayed a permanent, physiological low, methionine uptake.

Wilmshurst et al. observed increased spinal cord FDG uptake following radiotherapy, without any neurological signs (Wilmshurst et al. 2000). Their patient was irradiated for a recurrent, previously resected low grade cauda equina ependymoma (the radiation dosage was not stated). Two months after irradiation, FDG PET investigation revealed an increased tracer uptake (SUV 5.2); no sign of tumor was detected by MRI during the subsequent 3-year clinical course. The difference in absolute postirradiation values between our findings and those of Wilmshurst et al. may be explained by several factors. The doses applied most likely were dissimilar: the spinal cord of our patient received only 40 Gy, while there is a strong likelihood that the cauda equina tumor required a higher radiation dose. Although the radiobiological investigations of our patient did not reveal unequivocal signs of an increased radiosensitivity, a possible difference in the individual responses to radiation cannot be *ab ovo* ruled out. Finally, the exact time protocol of the FDG imaging was not the same in the two cases. It is noteworthy that an increased FDG uptake in the brain was earlier reported following intensive radiotherapy of malignant brain tumors (Janus et al. 1993).

The concordant results allow speculation concerning the pathomechanism of the transiently increased FDG accumulation within the irradiated spinal cord. An elevated glucose metabolic rate can be concomitant with cell division or with inflammatory processes that may comprise or be part of the pathological responses of the CNS to ionizing radiation (Godwin-Austen et al. 1975; Jellinger and Sturm 1971; Kristensson et al. 1967; Palmer 1972; Reagan et al. 1968; Schultheiss et al. 1988; Schultheiss et al. 1995). Demyelination (due to the death of oligodendrocytes and glial progenitor cells) is one of these pathological processes and it inevitably results in consecutive gliosis and

astrocytosis (Type 1 lesion). Both cellular reactions require extra energy consumption. It is generally accepted that energy-demanding remyelination is a *sine qua non* prerequisite for functional regeneration (Keirstead and Blakemore 1999). Jeffery reported normalization of a toxin-induced locomotor deficit of the spinal cord accompanied by spontaneous remyelination (Jeffery and Blakemore 1997). Recent studies have revealed that many regions of the adult CNS contain pluripotent progenitor elements that have the ability to generate new neurons and possibly glial cells (Yamamoto et al. 2001). Sasaki et al. described that transplantation of isolated bone marrow fraction resulted in the repair of X-ray-demyelinated adult rat spinal cord axons: apparently the bone marrow-derived stem cells underwent differentiation into myelin-forming cells (Sasaki et al. 2001). It has also been shown that the number of oligodendrocyte progenitor cells increases following mechanical spinal cord injury (Ishii et al. 2001).

Vascular (Type 2) lesions include endothelial cell damage, preferentially in capillaries and venulae, which is usually accompanied by a chronic inflammatory reaction involving mononuclear cells (lymphocytes, mainly T cells, macrophages) and microglia, simultaneously with fibrinoid necrosis of the vascular wall. This so-called radiation vasculitis results in an increased FDG uptake. Eventually, thickening of the vessel walls (hyaline change), telangiectasia and thrombosis/occlusion evolve. In addition to the white matter reactions, gray matter sequelae may also occur in the anterior and posterior horns of the spinal cord (Jellinger and Sturm 1971; Kristensson et al. 1967; Palmer 1972; Reagan et al. 1968; Sanyal et al. 1979), and it is not clear whether or not this may contribute to the elevated glucose metabolic rate.

Thus, the temporarily increased FDG uptake after radiotherapy may be related to subclinical, transitory demyelination and vascular inflammation, triggered by a not too

high dose of irradiation. The background level of $[^{11}C]$methionine accumulation in the spinal cord segment exposed to radiation can be regarded as strong evidence against intensive cell proliferation or intense inflammation. The almost complete disappearance of the temporary increase in the FDG uptake of the irradiated spinal cord region by the 44th month is in accord with the finding that the monkey spinal cord virtually completely recovers from radiation damage in 3 years (Ang et al. 2001).

b. *PET characterization of partially reversible radiogenic spinal cord injuries*

I. PERMANENT LHERMITTE'S SIGN

In a clonogenic assay, fibroblasts obtained from Patient 2 displayed much higher radiation sensitivity than those of healthy controls, while radiation sensitivity of the fibroblasts from Patient 3 was normal (Fig. 7b).
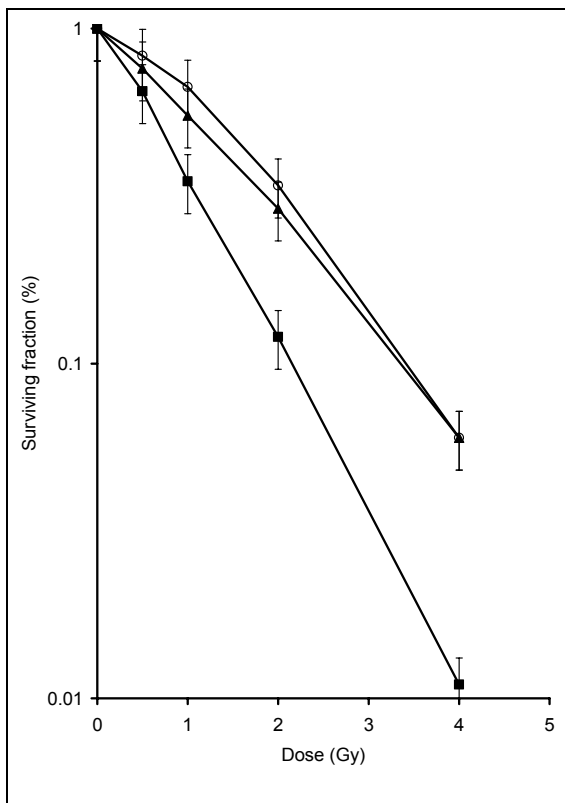

Fig. 7b *Survival curves with standard deviation for primary fibroblasts cells. Filled rectangles: fibroblasts from Case 1; filled triangles: fibroblasts from Case 2; open circles: average survival of fibroblasts from 6 healthy children.*

In the comet assay, no essential difference in DNA repair capacity was found between the patients' lymphocytes and those of the controls.

MRI evaluations did not reveal any pathological signs either in Patient 2 or 3 twenty-five and 7 years postirradiation, respectively (Fig. 8) despite the fact that conditions were completely appropriate to demonstrate a pronounced demyelinated state of the spinal cord, would that have been the case.
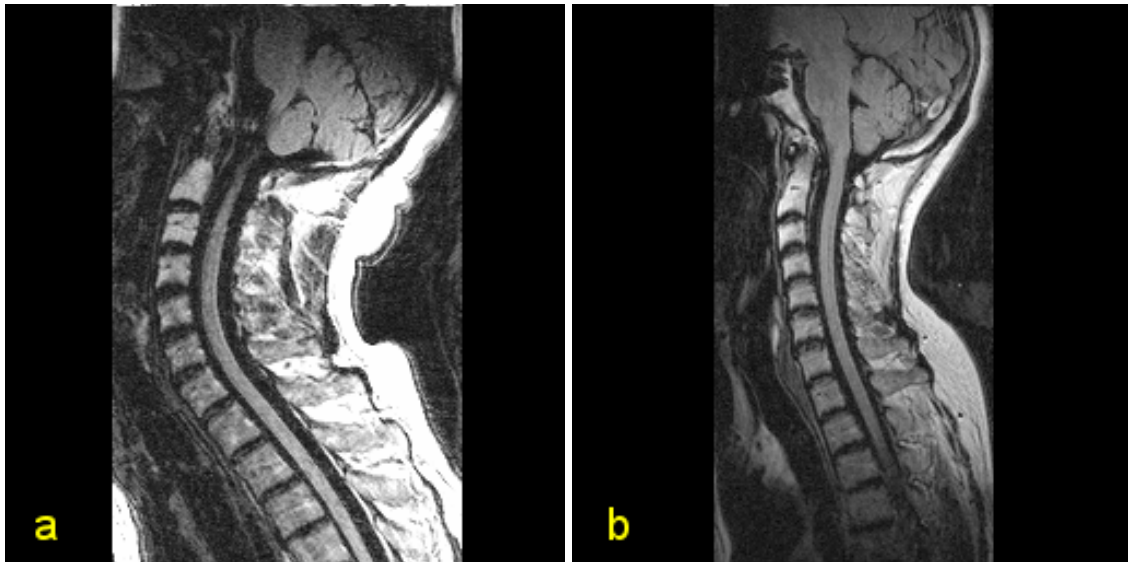
Fig. 8 *FLAIR MR images (median sagittal section of the head and neck region) of Patient 2 (a) and Patient 3 (b).*

A very low background FDG accumulation (mean SUV 0.84) was observed in the spinal cord of the reference subject, and the simultaneous $[^{15}O]$butanol uptake yielded perfusion patterns similar to that of the FDG accumulation map (Fig. 9).

Fig. 9 *PET examinations of a reference subject not exposed to irradiation (median sagittal section of the head and neck region) reveal low FDG, $[^{15}O]$butanol (BUT) and $[^{11}C]$methionine (MET) accumulation within the cervical spinal cord segments.*

The patient exhibited an extremely low [11C]methionine uptake within the cervical spinal cord with a mean SUV of 0.33 (for anatomical orientation: the vertebral processes display a yellowish light-blue descending l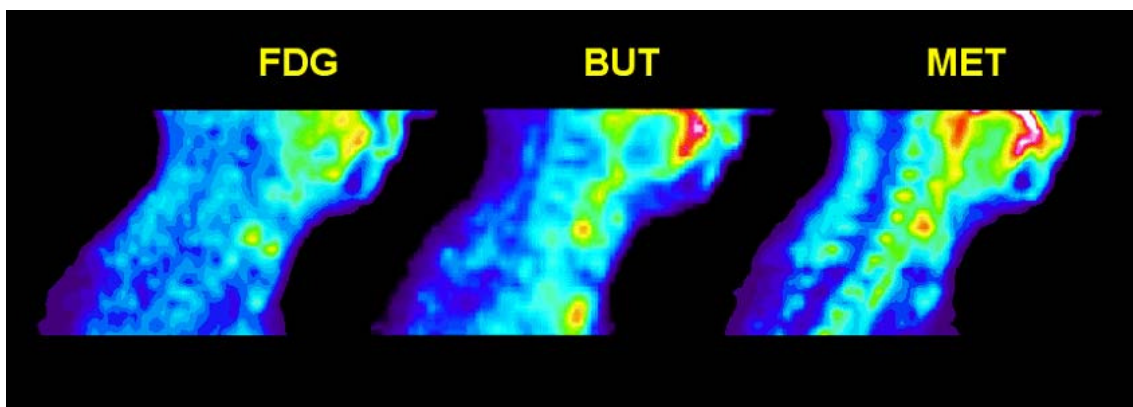ine in the posterior side of the neck, while the spinal cord appears as a dark-blue region with very low tracer uptake between this descending line and a parallel, anterior one with an increased tracer accumulation, corresponding to the vertebral bodies).

In Patient 2 and 3, FDG PET examinations (Figures 10 and 11) indicated an increased uptake in the irradiated cervical spinal cord (mean SUVs 1.56 and 1.60, respectively) as compared with the nonirradiated segments (mean SUVs 0.60 and 0.84).

Fig. 10 *PET examinations (median sagittal section of the head and neck region) of Patient 2 after cervical radiotherapy (biologically effective dose of 103.8 Gy$_2$).*

[15O]butanol PET investigation likewise revealed an increased blood flow in the region of the spinal cord that had previously been exposed to the radiation field, but [11C]methionine PET examination resulted in a negligible tracer uptake for both the irradiated (mean SUVs 0.36 and 0.31, respectively) and the nonirradiated segments (mean SUVs 0.50 and 0.40).
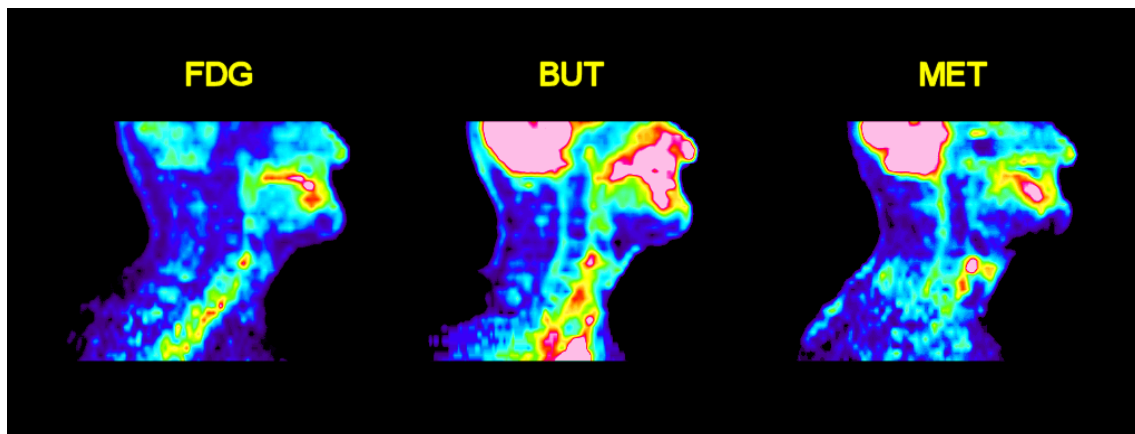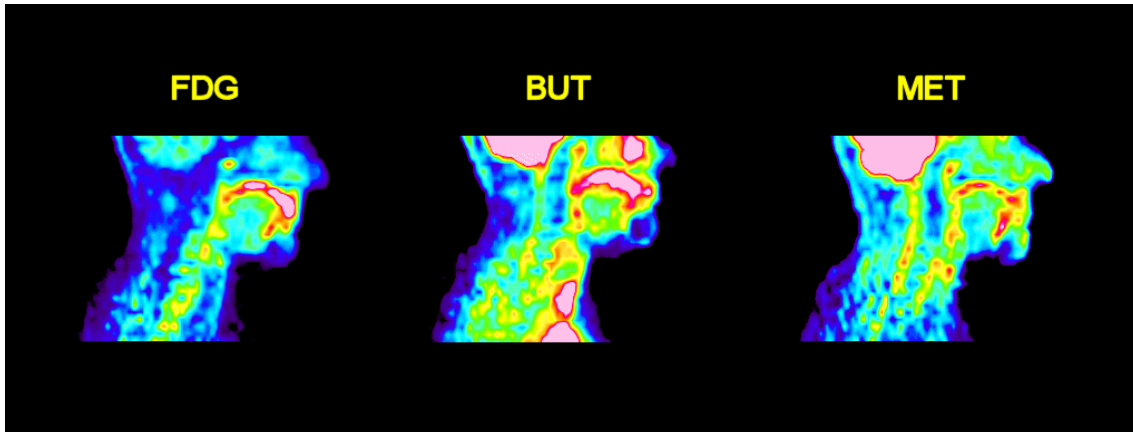
Fig. 11 *PET examinations (median sagittal section of the head and neck region) of Patient 3 after cervical radiotherapy (biologically effective dose of 94.8 Gy$_2$).*

The boundaries of the irradiated spinal cord segments can be found by localizing the irradiated cervical vertebral bodies and processes displaying a decreased butanol or FDG uptake relative to those outside the irradiated area, where accumulation of these tracers is much higher. The region corresponding to the irradiated segments C1 - C7 can also be seen in the methionine uptake panel because of definitely reduced tracer incorporation by the bone marrow of the irradiated vertebrae. No [^{11}C]methionine uptake was detected in the bone marrow of the vertebral bodies C1- C3 irradiated by high doses (69 Gy and 41.8 Gy, respectively).

Among the sensory radiogenic injuries of the spinal cord, Lhermitte's sign (LS) is the most frequent. It was first described in a soldier who had suffered a head injury during World War I (Marie and Chatelin 1917). Later on LS was characterized in conjunction with injuries and multiple sclerosis by Lhermitte (Lhermitte 1918; Lhermitte et al. 1924). He noted that the symptomatology was most probably secondary to damage to the cervical spinal cord resulting in demyelination. The clinical latency period corresponded to the normal survival of myelin, suggesting a possible interpretation, since damaged oligodendrocytes are not capable of myelin synthesis. As

the oligodendrocytes recover, myelin synthesis is resumed and the signs of the disease do not recur. The eponym "Lhermitte's sign" was first used by Read (Read 1932).

LS is characterized by a sensation similar to an electric shock passing down the spine in the cervico-caudal direction (it may also be felt in the upper and lower limbs). It is accompanied by a sense of intense pain, which is generally but not always symmetrical, but these signs never correspond in distribution with an anatomically defined territory of any spinal dermatome. LS develops following mostly flexion or less frequently after extension of the vertebral column (mainly its cervical part). This indicates the mechanosensitivity of the damaged (demyelinated) central and peripheral sensory axons.

LS is an early and typical sign of demyelination disorders, especially multiple sclerosis (Kachandani and Howe 1982), but also of other diseases, e.g. radiogenic myelopathy (Ballantyne 1975; Boden 1948; Carmel and Kaplan 1976; Fein et al. 1993; Jones 1964; Lewanski et al. 2000, Lossos and Siegal 1996; Mendenhall et al. 1982; Ševčikova et al. 2000; Thornton et al. 1991; Wen et al. 1992; Word et al. 1980), spinal cord trauma (Arasil and Tascioglu 1982; Chan and Steinbok 1984; Leaver and Loeser 1971, Lhermitte 1918; Marie and Chatelin 1917; Traynelis et al. 1990), epidural, subdural or intraspinal tumor (Newton and Rea 1996; Ventafridda et al. 1991) and spondylosis/discopathy (Signorini et al. 1984). It can be induced by chemotherapy, e.g. following intrathecal administration of platina and taxan compounds, cytosine arabinoside, busulfan and cyclophosphamide (Dewar et al. 1986; Eeles et al. 1986; Inbar et al. 1992; List and Kummet 1990; Lossos and Siegal 1996; van den Bent et al. 1998; Walther et al. 1987; Wen et al. 1992). LS may also be brought about by vitamin B12 (Butler et al. 1981) or thiamine deficiency (Lossos and Siegal 1996), low-dose pyridoxine toxicity (Parry and Bredesen 1985), nitrous oxide abuse (Blanco and Peters

1983; Layzer 1978), cystinuria (Blackburn and McLeod 1977), Behcet's disease (Page and Spiteri 1982) and herpes zoster (Vollmer et al. 1991).

LS was first reported as a phenomenon related to radiation myelopathy in 1948 (Boden 1948). In 1964 it was eloquently described, together with its temporal relationship to irradiation and its possible pathogenesis (Jones 1964). As opposed to multiple sclerosis, LS related to other causes is a transient, self-limiting, benign disorder. The radiogenic cases typically manifest 3 months following radiotherapy and gradually disappear within 6 months (Boden 1948; Jones 1964). In 2 autopsied cases, Jones did not find any signs of pathological alterations in the cervical spinal cord using conventional morphological/histopathological methods (Jones 1964). Only the rare form of LS, that occurs after a longer latency period, seems to forecast permanent spinal cord injury (Boden 1948; Carmel and Kaplan 1976; Jones 1964). There have been only a few studies on large enough patient groups with LS. These demonstrated an incidence of 3.6-13%, related mainly either to mantle irradiation (Carmel and Kaplan 1976; Ševčikova et al. 2000; Word et al. 1980) or radiotherapy of head and neck tumors (Fein et al. 1993; Jones 1964). A thorough research of the relevant databases has revealed only a single LS case described in the English literature with extraordinarily severe symptoms lasting for 1 year (Thornton et al. 1991).

According to the Common Toxicity Criteria Version 2.0 (National Cancer Institute 1997), our patients initially had Grade 3 (Patient 2) and Grade 2 (Patient 3) sequelae. The most noteworthy features of the clinical courses of the patients were (i) complete clinical recovery from the motor injuries (Patient 2), (ii) the incomplete regeneration of the sensory symptoms (Patient 2); and (iii) the permanency of the LS (both cases). A high total physical dose and large daily fractions are known to be the most important factors inducing the development of late motor or sensory spinal cord

injury (Schultheiss et al. 1995). Wong did not find radiation-induced white matter damage below a BED of 128 Gy_2 (Wong et al. 1994) in his patient group; thus, for our patients the calculated BED of 103.4 and 94.8 Gy_2, respectively, should not have created a substantial risk of late motor and sensory losses.

Although LS belongs to the late complications of spinal cord radiotherapy, it exhibits a substantially different dose-effect curve from the motor or other sensory sequelae. The development of this sign starts at around 30 Gy (Boden 1948; Jones 1964), and the most important risk factors for its manifestation (Fein et al. 1993; Lewanski et al. 2000) are a total dose of ≥50 Gy and a daily dose of ≥2 Gy (equivalent to a calculated critical BED of 100 Gy_2). The probability of occurrence of LS shows a shallow dose-effect curve (Fein et al. 1993). The incidence rate of LS is 3.3% or 8% with a total dose of <50 Gy or ≥50 Gy, respectively, and that with a fraction dose of <2 Gy or ≥2 Gy is 3.4% or 10%, respectively. These doses were exceeded and thus the enhanced risk of developing LS was evidently present in both of our cases.

MRI, a sensitive tool for detection of demyelination, earlier revealed signs of demyelination of the posterior cervical column only in multiple sclerosis (Gutrecht et al. 1993) and in a single case of LS related to an intraspinal tumor (Newton and Rea 1996). It has failed to show any pathological sign accompanying LS caused by other disorders (Lossos and Siegal 1996; Traynelis et al. 1990; van den Bent et al. 1998), and we assume that these normal MRI findings may be explained by the transient nature of LS. Since we did not detect any pathologic MRI sign in our patients even with long-standing LS (Fig. 8), we looked for another method. Inasmuch as PET is a very sensitive tool for assessing the functional status of different organs, we thought it might be of help in diagnosing permanent radiation myelopathy.

Few studies have been published on PET investigations of the spinal cord. This may be in part due to the low spatial resolution of PET cameras. The spinal cord normally has a very low FDG uptake (Fig. 9), because it is comprised of a high proportion of white matter with low glucose consumption relative to the small bulk of the gray matter (Di Chiro et al. 1983; Kamoto et al. 1998; Meltzer et al. 1998; Wilmshurst et al. 2000; Woesler et al. 1997). The spinal cord usually exhibits a rather low methionine uptake (Fig. 9), in consequence of the slow cell turnover (Higano et al. 1990; Sasajima et al. 1996; Wilmshurst et al. 2000). Irreversible injuries of spinal cord caused by radiation have not as yet been studied by PET. Difficulties inherent in such studies include the quandary of transportation of these patients and the low likelihood of acquiring clinically relevant information.

We studied a case (Patient 4) of partially reversible radiation myelopathy by PET (Ésik et al. 1999). In our report on that case, which to the best of our knowledge is the only one that explored regional tissue perfusion of the spinal cord, we described that recovery from radiogenic myelopathy was manifested by increased FDG and [^{15}O]butanol uptakes, but a negligible [^{11}C]methionine accumulation. Our data imply a close direct relationship (coupling) between regional spinal cord blood flow and glucose metabolism. In the present cases with an almost completely redeemed radiation myelopathy, we observed a similar increase of FDG and [^{15}O]butanol accumulation, but negligible [^{11}C]methinonine uptake within the irradiated region.

As mentioned earlier, an elevated glucose metabolic rate can indicate either active cell proliferation and/or inflammation. The irradiated part of the spinal cord, however, did not exhibit a higher than background methionine accumulation, which is a convincing and substantial evidence for lack of any significant cell proliferation. This is consistent with what can be expected: there is no basis to anticipate that following

stabilization and consequent improvement of the clinical status throughout several years there would still be a restoration process involving intensive cell proliferation.

Similarly, we are inclined to assume, that years after the occurrence of the lesion, inflammation would cease and would not make a substantial (if any) contribution to the increase in metabolic activity. The results of pathological studies (Jellinger and Sturm 1971; Palmer 1972; Schultheiss et al. 1988 and 1995) also argue against a significant inflammatory reaction in radiation myelopathy. Decreased cerebral FDG uptake (Valk and Dillon 1991) observed following high-dose radiation may support the argument that any inflammatory and microglial or astrocytic reactions within the spinal cord would not cause considerable increase in FDG accumulation. Thus, the explanation for increased glucose uptake should be related to other phenomena that require augmented energy consumption.

Radiation damage brings about alterations in the molecular structure of the axon membrane and demyelination is one of the most marked changes (Jellinger and Sturm 1971; Kristensson et al. 1967; Palmer 1972; Reagan et al. 1968; Schultheiss et al. 1988 and 1995). After loss of the myelin sheath, the segments between the nodes of Ranvier, harboring sodium channels in low density, are exposed to the interstitial fluid. Demyelization of the axons results in a reduced speed of the action potential conduction. It is documented that a higher than normal density of sodium channels may restore conduction in some chronically demyelinated axons (Felts et al. 1997; Waxman 1998). It is fair to speculate that the modified molecular structure and altered conducting mechanisms of these internodal segments give rise to extra energy requirements. This is most likely related to a larger perturbation of intraaxonal ion concentrations during the propagation of the action potential as a consequence of the larger number of sodium channels involved in conduction. The pumping-out of extra amounts of intracellular

sodium against a concentration gradient can be accomplished only at the cost of extra energy consumption.

The existing close coupling of glucose utilization to tissue perfusion in the spinal cord with its restored conduction provides a full explanation for the observed increased blood flow in the radiation-damaged segments with their reconstituted function and increased glucose metabolism. Such an interrelationship (i.e., the flow and glucose metabolism coupling has become an "axiom") is basically the same as that already proved for the brain (Roland 1993).

The most interesting finding was that the nearly complete recovery of the motor function (Patient 2) was accompanied by restoration of the sensory function. The residual deficits were not extensive; impairment of the motor function was subliminal and could only be detected only by ENG measurements. Supraliminal sensory deficits may be explained by the specific cortical projections of the sensation. The lack of realization of a very short disturbance of the motor function corresponding to the instantaneous electric shock-like sensation may be due to the fact that motor disorders of millisecond duration most likely are below the threshold of consciousness. Assuming that these mechanisms are involved, all the above findings are in accord with the unspecific (statistical) effect of ionizing radiation, that produces similar injuries in motor and sensory fibers.

A common feature of the clinical history of Patient 2 and Patient 3 was that their radiation-induced LS has persisted for many years, in contrast to the usual 6 months' long recovery. It is known that about 15% of the population has an increased radiosensitivity (Busch 1994), potentiating radiation induced damage. Thus, the question arises whether individual radiosensitivity presumably markedly surpassing the average in Patient 2 and Patient 3 might have contributed to the rare phenomena

observed. By the same token, it may also be of interest to know whether the differences in the extent of the radiation injuries of the two patients are related to differences in radiosensitivity, gender or age.

The levels of tumor radiosensitivity have been found to be independent of patient gender and age (StausbØl-GrØn and Overgaard 1999). Age has been reported to have no impact on the acute or late toxicity of curative thoracic radiotherapy (Pignon et al. 1998). Only insufficient data are available for an unequivocal judgment as to whether the radiosensitivities of neoplastic and normal tissues are comparable, but a recent review of published data suggests the possible existence of such a correlation (Bentzen 1997). These observations lead us to think that the difference in the extent of radiation-induced damage and the recovery between Patients 2 and 3 is not related to the gender and/or age difference between the two patients.

There is evidence indicating that individual radiosensitivity can contribute to the development of radiation-induced toxic reactions (Hall 2000; Hendry 2001). A strong correlation was found (Brock et al. 1995) between the clonogenic survival of cultured fibroblasts (that correlates with the radiation-induced DNA double-strand breaks in cultured fibroblasts) and the late radiotherapy reactions of breast cancer patients (Kiltie et al. 1999). The measured fibroblast radiosensitivity of Patient 2 was higher than that of Patient 3, whose fibroblasts displayed an average radiosensitivity. This suggests that the latter fact may have contributed to the differences between the two cases (more severe sequelae and longer case history of Patient 2). This seems probable, although we are aware of the fact that the degree of radiosensitivity of the fibroblasts may be used with caution and only as an estimate for that of oligodendrocytes or endothelial cells, which are thought to be targets of radiation damage to the CNS (Hall 2000).

II. INCOMPLETE SPINAL CORD TRANSECTION

In Patient 4 sensory impairment started to develop after a latency period of 1 month following the completion of radiotherapy, and motor sequelae with a latency of 5 months. The symptoms culminated at 9 months, and the incomplete cervical transection that evolved caused spastic paraplegia in the lower extremities, spastic and flaccid paresis in the left arm and sensory losses below the cervico-thoracic junction on the left, and below the 10th thoracic dermatome on the right side. The patient experienced difficulties in initiating urination and defecation. A gradual improvement of these deficits started 11 months after the completion of radiotherapy, and continued until her demise (the direct cause of death was unrelated to the above described symptoms). The patient was ambulatory, but with spastic paraparesis in the legs, some residual sensory deficits and constipation. During the early years of the disease, she received B1 and B6 vitamins to promote neuro-regeneration and piracetam to enhance microcirculation, neural transmission and neuro-regeneration.

During the acute phase of the neurological symptoms, only lumbar MRI was performed (due to misdiagnosis of the disease), but this did not demonstrate any pathological alteration. Diagnostic imaging of the appropriate part of the spinal cord was first performed 4 years following the completion of radiotherapy. The native T1 weighted MRI images revealed atrophy throughout the length of the spinal cord, reflecting axonal loss and Wallerian degeneration; it was especially marked in the upper two-thirds of the thoracic region (Fig. 12).
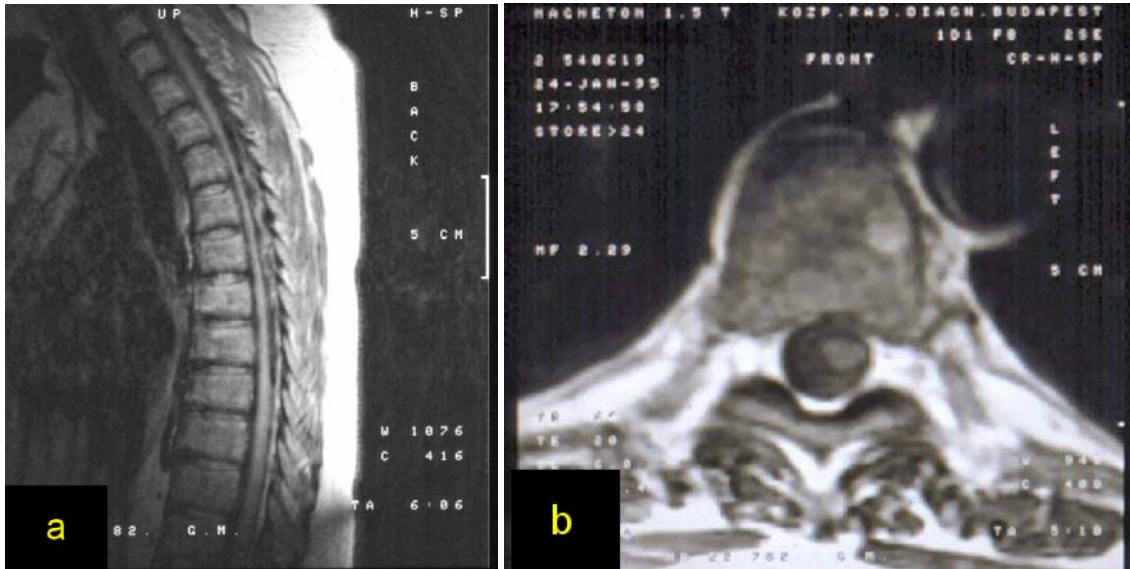
Fig. 12 *Intermediate-weighted MRI images showing spinal cord atrophy on the thoracic region in the midsagittal plane (a) and on the transaxial section at the level of the 6th thoracic vertebra (b).*

The first PET examination, during the 6th year of the clinical course, indicated an increased FDG uptake in the cervical spinal cord (Fig. 13).
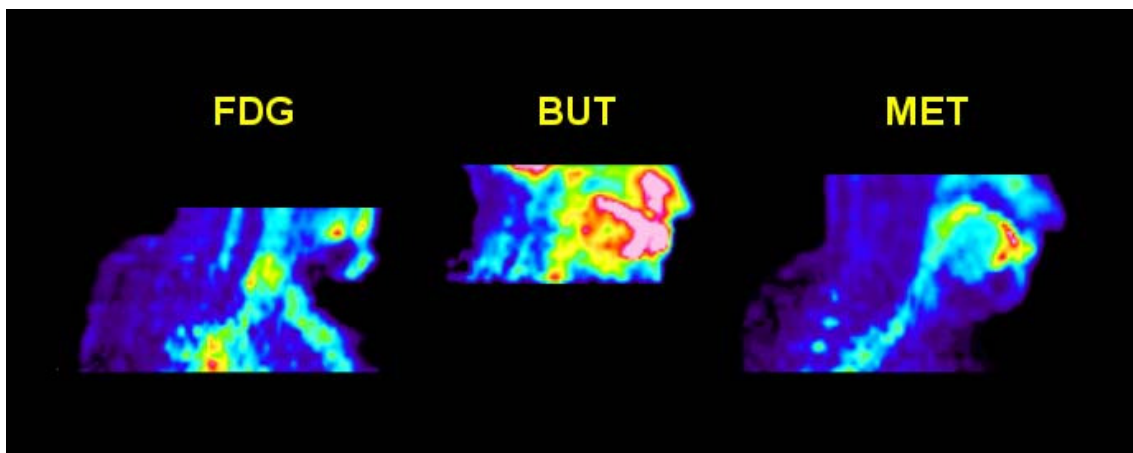
Fig. 13 *PET examinations (median sagittal section of the head and neck region) reveal increased FDG and [^{15}O]butanol, but low [^{11}C]methionine accumulation within the irradiated cervical spinal cord segments. The BUT and MET panels are shifted relative to the FDG panel in order to ensure the same vertical location of the identical anatomical structures in all three images.*

The FDG uptake in the irradiated cervical spinal cord region was found to be increased when compared to that of the unirradiated spinal cord segments. The ratio of the irradiated to the non-irradiated spinal cord standard uptake was 2.7. Eight months later, a second FDG PET examination revealed an unchanged longitudinal extent of the

increased uptake zone, with an irradiated to non-irradiated spinal cord FDG uptake ratio of 2.6. During the 8th year of the clinical course, [^{15}O]butanol PET examination showed an increased blood flow within the irradiated spinal cord segment, but [^{11}C]methionine PET examination indicated a negligible tracer uptake for both the irradiated and the non-irradiated segments of the spinal cord.

A BED of 165 Gy$_2$ was calculated for our patient. As radiation-induced injury occurs with increasing probability at BEDs above 128 Gy$_2$ (Wong et al. 1994), the development of radiation myelopathy in our patient could easily be accounted for.

Although a marked improvement occurred in the sensory losses, the most noteworthy feature of the clinical course of this patient was the partial recovery of the motor impairments attributed to the white matter injuries. In contrast to the frequent recovery from sensory losses, recovery from motor sequelae is a rare event in radiation myelopathy: only 6 well-documented cases (Dynes and Smedal 1960; Glanzmann et al. 1976; Solheim 1971) have so far been published (Table 5), excluding the present case.

Table 5. Reported cases of radiation myelopathy with reversible motor symptoms

Author/ case no.*	Estimated radiation dose to the spinal cord (R/cGy/BED)	Latency period from completion of radiotherapy (months)	Motor symptoms at culmination	End of regression (years)	Residual motor signs
Solheim 1	5200 - 7900 R	19	quadriparesis	5	no
Solheim 2	5000 R	15	hemiparesis on the right	3.5	right arm weakness
Solheim 3	4800 R	5	paraparesis in legs	4	weakness in one leg
Solheim 4	5000 ±500 R	6	quadriparesis	7	weakness in legs
Glanzmann 4	4500 cGy	24	paraparesis in legs	not stated	weakness in legs
Dynes 10	6000 R	19	paraparesis in legs	intercurrent death	improvement
Present case	165 Gy$_2$	9	paraplegia in legs, paresis in left arm	intercurrent death	para- paresis in legs

* Numbers are case numbers within the published cohort.
Abbreviations:
R: Roentgens (not reported in Gy)
BED: biologically equivalent dose in Gy$_2$

Instead of a decreased FDG uptake of the spinal cord region exposed to high-dose irradiation, as had been expected on the basis of brain studies related to radiation injury, we observed an increased FDG accumulation in the irradiated region. Intensive cell division was excluded as an explanation for this finding based on the very low methionine accumulation (Roland 1993). The latter finding was similar to the previous cases. This is consistent with expectations, as one would not anticipate that a 9.5-year uninterrupted improvement in the clinical status would be followed by a restoration process accompanied by intensive cell proliferation.

The severity of the neurological deficits observed in our patient during the culmination of the symptoms implied extensive degeneration of the spinal cord, probably involving the entire white matter of the irradiated region. The observed partial recovery of all impaired nerve tracts permits the assumption that a certain degree of regeneration involved the entire cervical white matter, a conclusion in accord with the marked increase in FDG accumulation.

The observed increased blood flow in the radiation-damaged segments with reconstituted function is fully explained by the interrelationship between glucose consumption and tissue perfusion for the spinal cord with restored conduction and increased glucose metabolism.

As it was detailed previously in connection with the cases of permanent Lhermitte's sign, intense inflammation could be ruled out as a cause for the increased glucose consumption. The explanation could be the same as proposed for those two cases. Further support was acquired from the detailed post mortem pathologic analysis of the spinal cord.

c. *Autopsy findings in partially reversible radiation myelopathy*

Besides acute purulent meningitis throughout the CNS, the autopsy of Patient 4 did not reveal signs of any other illnesses. No sign of recurrent thyroid cancer was seen. The primary radiogenic injury was limited to the irradiated segments of the cervical cord and medulla oblongata. Grossly, extreme atrophy and bizarre distortion of the spinal cord were observed, accompanied by marked fibrosis of the meninges and dura mater.

The histopathology verified the macroscopically diagnosed acute purulent meningitis, revealing extensive perivascular and meningeal polymorphonuclear neutrophilic infiltration all over the entire CNS. There was no indication of chronic

inflammation, neither that of astrocytosis, gliosis nor an accumulation of mononuclear cells was detected. The cervical portion of the spinal cord that had received the miscalculated high dose of radiation displayed a pronounced, bilateral loss of myelin and axons mainly within the lateral and to a lesser degree within the posterior columns (Fig. 14a-c).
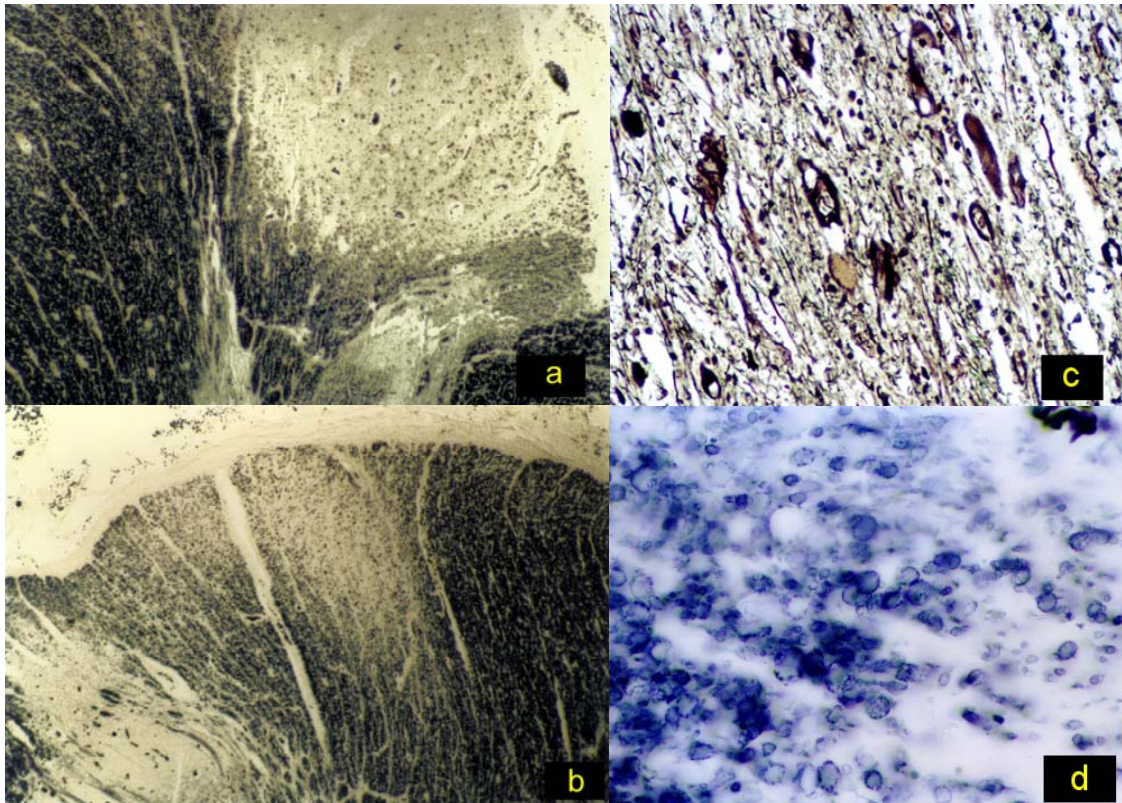
Fig. 14a-d *Extensive demyelinization in the lateral (a) and posterior (b) columns of the cervical spinal cord (Woelcke myelin staining) and resultant axonal loss (c, Bielschowski staining). Incipient, partly remyelinated sheaths are suggestive of early regeneration at the edges of the demyelinated plaques (d, Woelcke myelin staining).*

A few thinly remyelinated sheaths were present at the edges of the demyelinated areas (Fig. 14d). Moreover, bilateral, extensive damage to neurons was also observed, most markedly among the motor neurons of levels C1 and C2 (Fig. 15), and was practically identical on both sides.
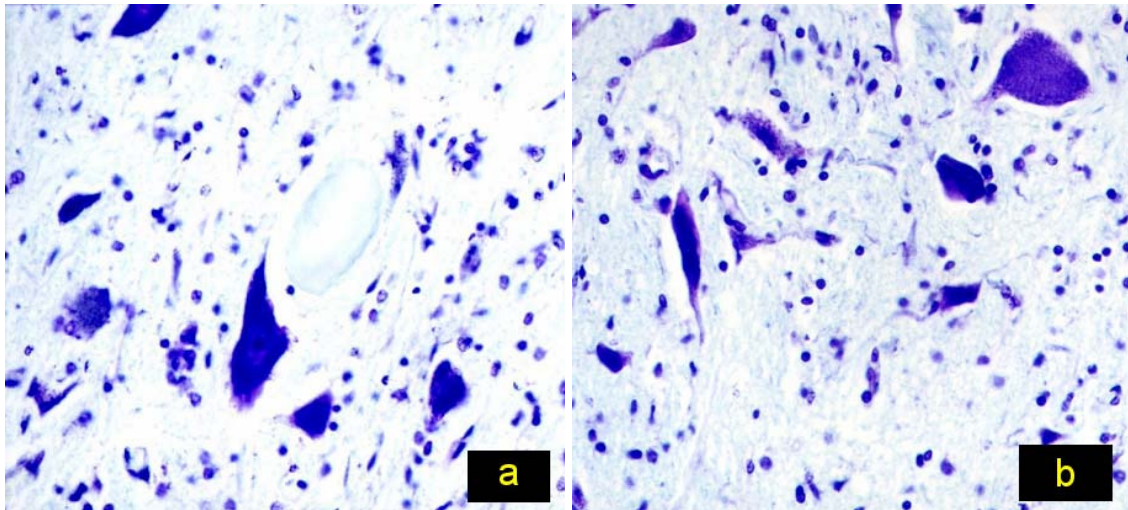
Fig. 15 *Motor neuron degeneration (karyolysis, chromatolysis, coarse appearance of the Nissl substance) in the right (a) and left (b) anterior horns at levels C1 and C2 (Nissl staining).*

The larger and smaller arterioles and venules of the irradiated spinal cord were intact (Fig. 16a,b), but the walls of some capillaries exhibited thickening (Fig. 16c).
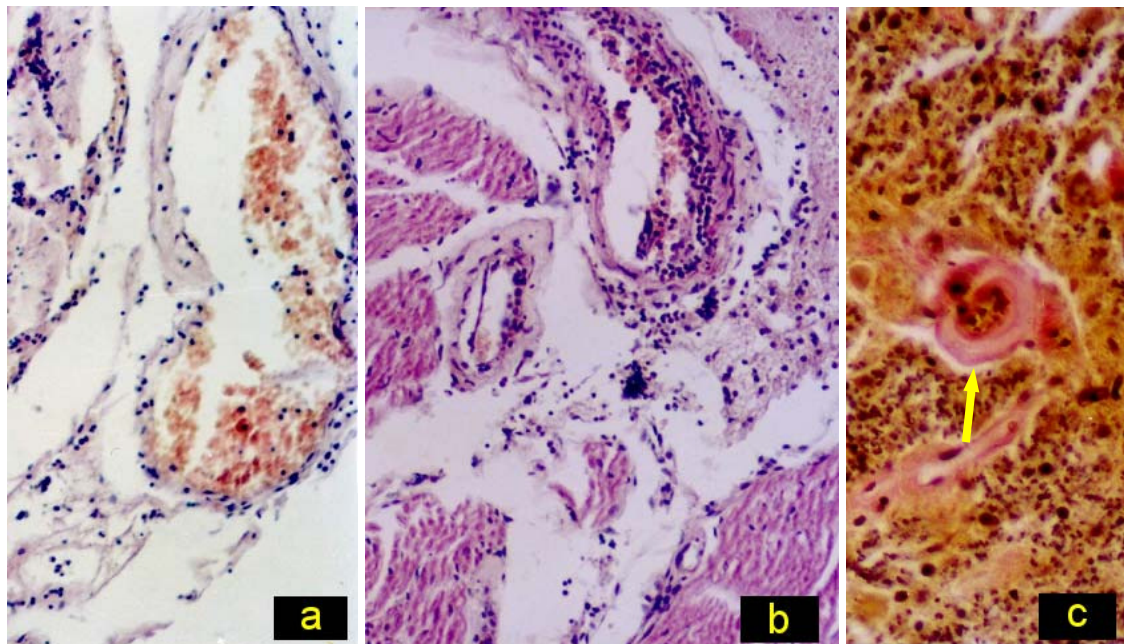
Fig. 16 *Larger (a) and small (b) vessels of the irradiated spinal cord (haematoxylin-eosin staining). Leukocytic infiltration around the vessels and within the meninges represent acute purulent meningitis. Thickening of the capillary walls (c) is demonstrated by van Gieson staining (arrow).*

Telangiectasia was not observed. Below the irradiated cervical spinal cord segments, bilateral, secondary pyramidal tract degeneration was obvious (Fig. 17).
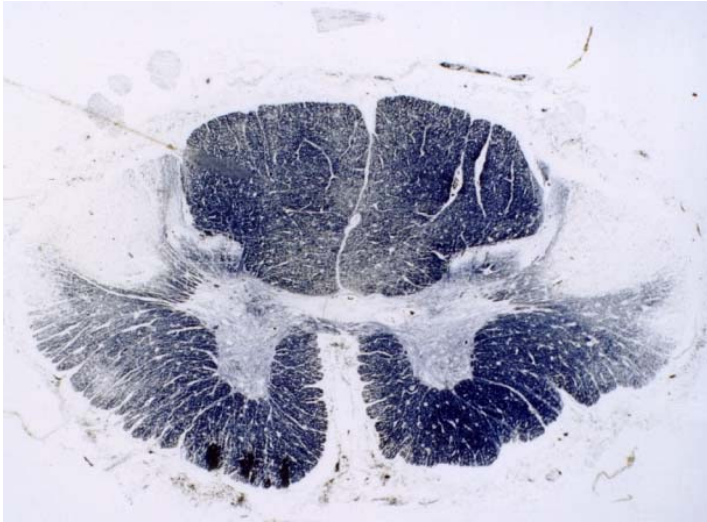
Fig. 17 *Bilateral, secondary degeneration of the pyramidal tract at the mid-thoracic level (Woelcke myelin staining).*

The medulla oblongata exhibited patchy demyelination and neuronal degeneration (especially in the dorso-medullary region that involves the respiratory center); this was most probably caused by the erroneously delivered high radiation dose at the margin of the cervical radiation fields, and this probably was responsible for the clinically unexplained central apnea. Although the demise of the patient was not directly related to this radiation-induced side-effect, the radiation injury causing central apnea incapacitated her and the bed-ridden patient finally fell for the presumably iatrogenic purulent CNS infection that was the direct cause of death.

Tables 6 and 7 present data on 17 cases of Type 1 lesions and 10 cases of Type 2 injuries, collected from the English literature (Jellinger and Sturm 1971; Kristensson et al. 1967; Palmer 1972; Schultheiss et al 1988; Ésik et al. 1999; Cohen et al 1983; Alfonso et al. 1997; Black et al. 1980; Burns et al. 1972; Coy P and Dolman 1971; Escò et al. 1993; Koehler et al. 1996; Komachi et al. 1995; Lampert and Davis 1964; Marty and Minckler 1973). Type 2 lesions were further subclassified on the basis of the delivered dose, as cases with doses in the conventional therapeutic range (6 cases) and those with an accidental, extremely high overdosage (4 cases).

Table 6. Characteristics of autopsied radiation myelopathic (Type 1) cases with extensive demyelination and no/mild vascular lesions

Author	Case no.*/ Affected region	Total dose/ fraction dose of spinal cord (Gy)	Latency period of neurol. symptoms (months)	Survival following completion of first radiotherapy (months)	Degree of motor neuron degeneration	Dominant vacular lesions	Known additional predispos- ing factors**
Black	1 / C	60/n.s.	9	<12	considerable	V	73 ys
Burns	1 / C	107/2	1 (R, 10 mo)	15	varying	V	57 ys
	2 / C	18/2+45/4.5	7 (R, 17 mo)	28	considerable	V	O_2
	3 / C	42/4.5	16	17	varying	V	O_2, 59 ys
	4 / C	118/~2	9 (R, 10 mo)	20	varying	V	61 ys
Cohen	1 / CTL	50/2	2 (R, 60 mo)	102	varying	no	chemo
Coy	2 / T	39/4.9	10	12	considerable	V	O_2, 53 ys
	3 / T	39/4.9	30	44	no	no	O_2, 63 ys
Ésik	1 / C	61/3.4	5	120	considerable	no	no
Jellinger	1 / C	19-39/n.s.	3	6.5	rel. pres.	no	no
	5 / C	24-33/n.s.	3 (R, 6 mo)	12	rel. pres.	no	51 ys
Kristensson	1 / C	<51/n.s.	40	48	varying	V	67 ys
Lampert	1 / C	57/n.s.	3	3.5	no	V	64 ys
Palmer	2 / C	42/n.s.	19	24	rel. pres.	V	63 ys
	6 / TL	78/n.s.	8 (R, 6 mo)	24	rel. pres.	no	O_2
Schultheiss	1 / T	n.s.	7	12	n.s.	no	n.s.
	2 / C	n.s.	12	15	n.s.	no	n.s.

Abbreviations/remarks:
* In the original publication
**Age indicated only above 50 years
C = cervical
L = lumbar
mo = months
n.s. = not stated
O_2 = hyperbaric oxygen inhalation
R = reirradiation (with elapsed time)
rel. pres. = relatively preserved
T = thoracic
V = vasculitis (vessel wall thickening)

Table 7. Characteristics of autopsied radiation myelopathic (Type 2) cases with extensive vascular lesions and no/mild demyelination

Author	Case no.*/ Affected region	Total dose/ fraction dose of spinal cord (Gy)	Latency period of neurol. symptoms (months)	Survival following completion of first radiotherapy (months)	Degree of motor neuron degeneration	Dominant vascular lesions	Known additional predispos- ing factors**
Therapeutic dose range							
Koehler	1 / C	46/2.25	7	13	n.s.	V, N	72 ys diab. mell. hypertension
Komachi	1 / T	70/2	48	56	yes	V, N	81 ys
Marty	1 / C	55/1.4	48	50	n.s.	V, O, N	77 ys
Palmer	1 / T	41/5.3-5.6	13	15	rel. pres.	N	chemoth.
	4 / C	55/1.75	48	50	rel. pres.	N	77 ys
	7 / C	30/3.75	17	20	rel. pres.	N	63 ys chemoth.
Accidental dose range							
Alfonso	2 / C	120/20	2	3	n.s.	V, O, N	-
	4 / C	180/20	1.5	3	n.s.	V, O, N	60 ys
	5 / C	140/20	1	1.5	n.s.	V, O, N	54 ys
	6 / C	160/20	1	1.5	n.s.	V, O, N	58 ys

Abbreviations/remarks:
* In the original publication
**Age indicated only above 50 years.
C = cervical
N = necrosis
n.s. = not stated
O = obliteration
rel. pres.= relatively preserved
T = thoracic
V = vasculitis (vessel wall thickening)

The most striking difference between the two main types (i.e., Type 1 and Type 2, conventional radiotherapeutic dosage subtype) of radiation injury is observed in the length of the latency period preceding the time point when symptoms were first observed. The mean latency period for demyelination is 11 months and that for vascular injury is 30 months. This corroborates the data reviewed by Schultheiss, i.e., 14 and 29

months for the two groups, respectively (Schultheiss et al. 1988). At the same time, the average durations of survival of the patients following radiotherapy were almost identical: 30 months for Type 1 lesions and 34 months for Type 2 (therapeutic dose range subtype), as calculated from the data in Tables 6 and 7. The noteworthy difference in the average survival from the time of appearance of the symptoms might imply a less effective repair of the vascular damage. The specified fraction size or the total dose or both were too high (>2 Gy and >50 Gy, respectively) in all cases in both groups. Additional known risk factors (reirradiation, diabetes mellitus, hypertension, hyperbaric O_2 inhalation, chemotherapy and old age) were documented in most cases in the "demyelination group", and in all cases in the group characterized by dominantly vascular sequelae. Although the listed differences are distinct and the associated histopathologies differ appreciably, the exact pathogenetic factors and the determinants of the actual scenario for the development of a dominant white matter or a dominant vascular injury remain uncertain. The features of the clinical history of our case are characteristic of the group of predominantly demyelination injuries, as she had no additional risk factors, and there was a short latency period (5 months) and a long survival (9.5 years) following the appearance of the symptoms.

A special group of radiation myelopathic patients with extremely high fraction size (20 Gy) and exceedingly high total dose (>120 Gy), due to a malfunctioning linear accelerator, comprises a distinct clinical entity (Alfonso et al. 1997; Escò et al. 1993). The latency period in these patients was very short (around 1 month) and the disease had a very fast progression. The vascular injuries developed exceptionally quickly, and all patients died within 3 months, before the development of significant demyelination.

As expected from the clinical symptoms, we found extensive demyelination with axonal loss and marked lower motor neuron sequelae. It is worth pointing out that,

although the radiation dose was delivered homogeneously, as reflected by the practically homogeneous histopathological reaction on both sides, an obvious asymmetry appeared in certain clinical signs and symptoms (e.g. spasticity and flaccid paresis only in the left arm, side differences in severity of sensory symptoms, etc.). This may be related to the fact that radiation-induced destruction, i.e., cell loss of a given cell type, does not necessarily or inevitably result in a precisely predictable functional deficit. A decrease in the population of a single cell type is too simple a pathogenetic explanation to account for the development of a defined loss of function.

The almost complete lack of vascular injuries in our case deserves discussion. Although the vascular endothelium is one of the structures that are highly sensitive to irradiation, there might be a significant individual variation in this response (Safwat et al. 2002). Factors such as the age of the patient, the presence of hypertension, diabetes mellitus, other vascular diseases, the efficiency of the blood supply (e.g., developmental abnormalities), the endocrinological status, etc., may also be of importance. Our patient was a low-risk case for vascular injury from the point of view of additional predisposing factors, since she was relatively young (36 years) and, apart from her papillary thyroid cancer, she did not suffer from any illness during the 10-year long clinical course.

A single set of pathological data cannot provide direct evidence against recovery of the morphological and functional structure of the spinal cord from a damaged state that might have been more serious earlier than at the time of the autopsy. However, the pathological state revealed by the post mortem examination is completely concordant with the functional deficit observed with the culminating clinical symptoms. Thus, it could be concluded that the probability of an anatomical recovery explaining the partial restoration of the functional damage is low. This reasoning implies that functional

recovery may be supported by a mechanism that is different from that which had been destroyed by ionizing radiation.

Repeated PET investigations of Patient 4 demonstrated an increased FDG accumulation and $[^{15}O]$butanol perfusion, but a negligible $[^{11}C]$methionine uptake in the irradiated spinal cord segment.

In an attempt to interpret the clinical peculiarities in the case history of our patient, together with the results of the PET imaging and histopathology, we refer first of all to the gradual recovery of the functions lost due to radiotherapy. It is generally accepted that remyelination allows restoration of saltatory conduction and regaining of the normal function lost during demyelination. As we reported in connection with Patient 1, some authors claim that remyelination is a clear prerequisite to a sustained functional recovery (Keirstead and Blakemore 1999). This specific regeneration often fails in demyelinating diseases such as multiple sclerosis, though the consecutive functional deficit may get eliminated spontaneously. Recent studies have revealed that many regions of the adult CNS contain neuronal progenitors that have the ability to generate new neurones and glia (Chari and Blakemore 2002; Yamamoto et al. 2001). Several adult CNS regions exhibit neurotropic-factor responsiveness, including the spinal cord (Tuszynski and Gage 1995). Nieder et al. surveyed data relating to the pathogenesis of radiation myelopathy and suggested that the administration of certain cytokines may increase the proliferation of oligodendrocyte progenitors, upregulate the synthesis of myelin constituents and promote myelin regeneration in the adult CNS (Nieder et al. 1999). Moreover, neurons in several CNS regions extend neurites after injury when presented with growth-promoting substrates. It has also been shown that the number of oligodendrocyte progenitor cells increases following mechanical spinal

cord injury (Ishii et al. 2001). Thus, even the mature CNS is highly plastic when stimulated by appropriate triggers or signals.

The efficacy of induced differentiation and proliferation of progenitor cells must be extremely low, as judged on the basis of the high percentage of residual demyelinated axons in our case, revealed by the histopathology in the involved region of the spinal cord at the end of the 10-year case history. The length of the period of spontaneous remission and the far from complete repair of the injured axons argue vigorously against a significant cell proliferation rate related to this special regeneration process. Thus, intensive cell proliferation does not seem to be a plausible explanation for the high FDG accumulation. At the same time, this conclusion is strongly supported by the very low methionine uptake within the irradiated segments of the spinal cord. The subthreshold accumulation of this tracer is additionally concordant with the lack of intense chronic inflammation and/or astrocytosis and/or gliosis as revealed by autopsy. This argumentation, and the lack of any clinical sign of acute inflammation at the time of the PET imaging, rather reliably precludes any chronic inflammatory processes as a possible explanation for the elevated FDG uptake.

The microvasculature is a ubiquitous system, which often plays a major role in the pathogenesis of radiation damage to normal tissues. A late effect of irradiation is the manifestation of injury not only to the endothelium, but also to the basement membrane, smooth muscle cells and adventitial perivascular cells (Fajardo et al. 2001). There is an abnormal proliferation of endothelial cells, leading to vessels of irregular diameter and shape (Baker and Krochak 1989; Cromheecke et al. 1999). Archer described a pattern of progressive degenerative vascular changes in response to ionizing irradiation of the retina (Archer and Gardiner 1994), ranging from occlusion and microaneurysm formation to microvascular abnormalities such as telangiectasia. As a consequence, the

vascular supply to the irradiated structures was altered and resulted in a decreased perfusion. Accordingly, our previous PET data on increased perfusion, though unusual, are consistent with the present morphological observation of the lack of any remarkable sign of vasculopathy at the time of autopsy. This finding also reliably rules out the earlier existence of this progressive pathological process.

The autopsy revealed neuronal loss in the irradiated segments of the spinal cord, in accord with the detected flaccid paresis. The decrease in the number of viable and functioning cells argues for decreased and also against increased metabolic activity of the appropriate segments, and thus cannot explain the increased FDG accumulation.

The increased metabolic activity in the irradiated segment of the spinal cord cannot be accounted for either by intensive regenerating processes involving marked cell proliferation, or by vasculopathy or chronic inflammation since the autopsy showed lack of all these changes. However, as it was introduced in the previous cases, we suggest that all functional imaging findings can be interpreted as a consequence of altered conduction mechanisms of the action potential, involving an increased number of sodium channels along the demyelinated segments of the injured axons. During action potential propagation, the activity of these channels of higher than normal density in chronically demyelinated axons, induces a greater change in the intracellular sodium concentration, which in turn requires a larger amount of energy to maintain homeostasis. This interpretation is consistent with the autopsy results and is at least partially supported by the latter. This train of thought is corroborated by data from the literature (Felts et al. 1997; Waxman 1998).

d. *PET findings in radiogenic lower motor neuron disease*

Magnetic resonance imaging (MRI) of Patient 5 patient failed to show any pathological signs. The FDG PET examination indicated an increased uptake within the

lower thoracic and lumbar spinal cord (Fig. 18), while [11C]methionine PET investigation resulted in no tracer uptake in either the irradiated or the non-irradiated segments of the spinal cord. No methionine uptake was found in the D10-12 and lumbar vertebral bodies, in accordance with the boundaries of the radiation portal (Fig. 19). [15O]butanol PET examination did not provide interpretable results because of the high blood flow in the abdominal large vessels.
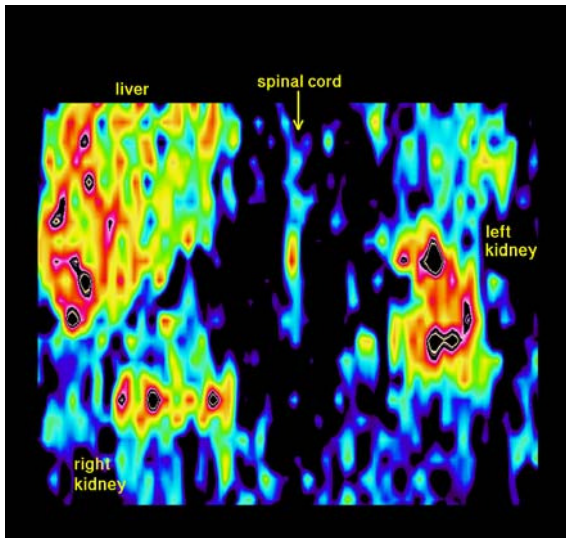
Fig. 18 *FDG PET examination (coronal section of the upper abdomen) reveals an increased FDG uptake within the irradiated lower thoracic and upper lumbar spinal cord segments.*
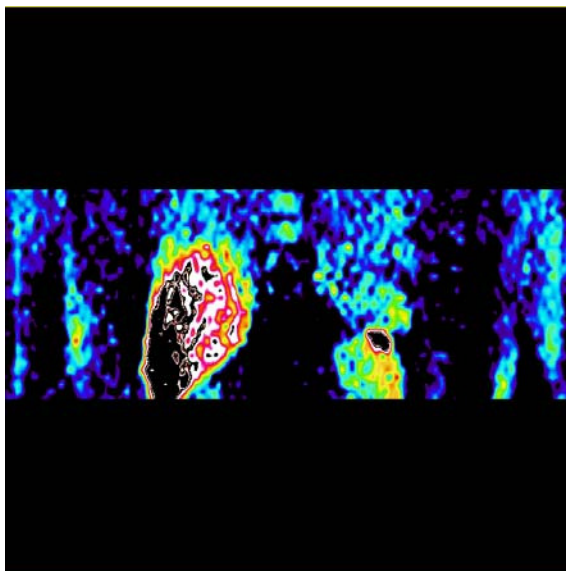
Fig. 19 *Methionine PET examination (coronal section of the upper abdomen) reveals no tracer uptake in the bone marrow of D10-12 and the lumbar vertebral bodies.*

The radiation sensitivity of the fibroblasts of the LMND patient was in the same range as that of the controls (about 80%, 60%, 28% and 5% clonogenic survival after irradiation with 0.5, 1.0, 2.0 and 4.0 Gy ^{60}Co-γ radiation, respectively). In the comet

assay, no essential differences in the initial damage were detected and the DNA repair was complete after 4 h. In the micronucleus assay, the spontaneous micronucleus frequency was slightly higher in the LMND patient (39 micronuclei versus 16±8 in 1000 binucleated cells) than in historical controls (Köteles et al. 1993). After irradiation with 2 Gy γ-radiation, the micronucleus frequency was elevated to 236/1000 cells, which was in the same range as the controls (Kormos and Köteles 1988).

In the chromosome aberration studies after *in vitro* irradiation of peripheral blood samples, the mitotic index was very low after phytohaemagglutinin induction and colcemid arrest. In two separate experiments, the scores in the unirradiated samples of the patient were only 41 and 100 metaphases, respectively. Meanwhile, the number of chromosome aberrations was very high (4% and 7% aberrations, respectively) compared to that of the controls (3%). In the first experiment, 1 acentric fragment, 1 minute chromosome, 1 deletion and 1 translocation were detected. In the second experiment, 1 cell contained dicentric and corresponding acentric fragments. In separate cells, we also scored 2 acentric fragments and 4 deletions. Unfortunately, we did not have a sufficient number of metaphasic cells after the 2-Gy irradiation.

The patient had a case history that was very similar to those published in the literature. He was 31 years old at the time of the diagnosis of his testicular cancer, and only flaccid paralysis developed in his lower extremities, without sensory or vegetative signs. To date, he is the only testicular cancer patient diagnosed with radiogenic LMND at the National Institute of Oncology (NIO), Budapest, although the NIO has been the center for the treatment of Hungarian testicular cancer patients since the early 1980s. During this period, about 7000 patients with seminoma and other types of testicular cancer (95% of all such cases in Hungary) have been treated there. All these patients

have been thoroughly followed up, and thus we can reinforce that radiation induced LMND really is a rare entity.

The noteworthy feature in the clinical course of this patient was the partial recovery from LMND. Recovery from grey or white matter damage causing motor deficit is a rare event: only 8 (Bowen et al. 1996; de Greve et al. 1984; Kristensen et al. 1977; SchiØdt and Kristensen 1978; Schold et al. 1979) and 7 (Ésik et al. 1999; Dynes and Smedal 1960; Glanzmann et al. 1976; Solheim 1971) well-documented cases, respectively, have been published. MRI does not seem to be an effective tool for the characterization of radiogenic LMNDs (Bowen et al. 1996; de Carolis et al. 1986; Feistner et al. 1989; Lamy et al. 1991; Tallaksen et al. 1997), since MRI consistently yields anatomical data regardless of existing disease that are similar to those obtained from studies done on healthy subjects.

Results of ENG measurements unequivocally refer to residual damage of the spinal cord still existing in partially recovered cases. While the values of the measured functional parameters of the sensory neurons and axons were identical to those characteristic for the physiological state, nerve conduction studies disclosed a decreased conduction velocity and diminished amplitude of the action potential. The simultaneous decrease of these values clearly indicates that the number of functioning axons has been reduced and segmental demyelination has occurred.

In the irradiated lower thoracic and lumbar spinal cord segments of our seminoma patient with LMND who exhibited a partial functional recovery, we observed an increased FDG uptake, but a negligible [11C]methinonine uptake (the [15O]butanol study could not be interpreted).

These findings are consistent with expectations, as it is not to be anticipated that a 15-year uninterrupted improvement and stabilization of the clinical state would be followed by a restoration process accompanied by intensive cell proliferation.

Similarly to the previous cases, we do not believe that inflammation makes a substantial contribution, if any, to the increase in metabolic activity detected 15 years after the occurrence of the lesion. Results of pathological studies argue against a substantial inflammatory reaction in LMND (Berlit and Schwechheimer 1987; Bowen et al. 1996; Schold et al. 1979).

Considering the above we can conclude, that in this case again the changes related to the restoration of the conduction on demyelinated axons might be responsible for the elevated energy demand, hence the increased FDG uptake.

During the radiotherapy of our patient, the total dose (44 Gy), the daily dose (2 Gy) and the BED (88 Gy$_2$) were below the usual threshold doses for radiation injury (Schultheiss et al. 1995), and we were not able to demonstrate any further obvious radiotherapeutic circumstances potentiating the development of any radiogenic complications.

Chemotherapy applied before radiotherapy may be a triggering factor with potential contribution to the development of radiation injury (Schultheiss et al. 1995). It is well documented, however, that intensive chemotherapy for a year is needed to diminish the threshold dose of radiogenic myelopathy in a combined therapy to 40.3 Gy (Chao et al. 1998). Our patient received only 1 conventional cycle of chemotherapy, and it therefore seems unlikely that this might have been a crucial factor. The worsening of the LMND following the second course of chemotherapy, however, indicates the role of toxic medication in the progression of the neurological sequelae. Interestingly, we detected an increased number of chromosome aberrations in this patient after 15 years.

The persistence of these aberrations might easily have been a long lasting consequence of the combined radio- and chemotherapy, although it cannot be proved beyond doubt.

The road accident and isoniazid-related peripheral neuropathy cannot be regarded as additional risk factors, since they had occurred about 10 years prior to the occurrence of the seminoma. The possibility of an increased individual radiosensitivity was ruled out by means of different radiobiological investigations. Thus, without evident predisposing factors for the development of radiogenic LMND in our case, we may hypothesize that it might have been related to some other additional precipitating factors, e.g., a subclinical viral infection or causes that we have not been able to clarify. The outlined interpretation is obviously somewhat speculative and its validation requires additional evidence.

e. *Suggested mechanism for the conduction of the recovered radiation-injured axons*

The axon's internodes normally contain very few sodium channels (Ritchie and Rogart 1977). Indeed, if sodium channels on a myelinated axon were evenly distributed over its entire length, their density would be much less than half of that present in most unmyelinated axons and would be too few to support conduction (Hirano and LLena 1995; Ritchie 1995; Querfurth et al. 1987). In order to develop and maintain continuous conduction in a *demyelinated* axon, the axon must form additional sodium channels. This is a prerequisite for the restoration of continuous conduction along a demyelinated axon. However, this alone will not ensure that conduction will occur, because a demyelinated axon has a giant capacitance charge (Waxman et al. 1995). This increased capacitance results in a huge increase in the amount of current required to depolarize its membrane to threshold. So the current passing down the axon from the last myelinated

region is normally insufficient to discharge a demyelinated membrane's capacitance to threshold.

This is more easily understood if one regards the axolemma and myelin, as the di-electric of a tubular capacitor. It separates the charged plates (the positive extracellular fluid from the axon's negative interior). By definition, capacitance is inversely proportional to the distance between the two plates of a capacitor. Therefore, the capacitance of a demyelinated axon is several folds higher than that of a myelinated axon, the numerous myelin layers of which insulate the negatively charged axon's interior from the positively charged extracellular fluid. Current that passes along the last myelinated segment to a demyelinated segment is mainly derived from the last node. On a large axon, this distance can be as far as 2 mm long, so the generated current is insufficient to depolarize the demyelinated membrane (axolemma) to threshold. Thus, conduction fails at the junction of the still myelinated and demyelinated segments, because the number of sodium channels in the demyelinated axolemma is insufficient for non-saltatory conduction. In the relevant literature, this conduction problem is referred to as impedance mismatch (Waxman 1978).

This conduction block is overcome by remyelination at the margins of the injured area and an increase in the number of sodium channels in the demyelinated axon (Sears and Bostock 1981). New myelin that results from remyelination has very short internodes, which allows summation of the current from several nodes. This increases the sodium current, overcoming the impedance mismatch and initiates continuous conduction in the demyelinated axon. This change, however, has several further consequences. The amount of sodium entering the axon with each impulse is greatly increased. The sodium-potassium pump cannot keep up with the high rates of sodium entry, when nerves are very active. Hence there is a "flooding" of the axon with sodium

that leads to the exhaustion of ATP supplies and conduction failure. Recovery time between impulses becomes prolonged and many impulses drop out, during fast trains of impulses.

The need of extra amounts of ATP to fuel the excessive task performed by the Na^+/K^+ pumps results in an elevated metabolic rate of glucose in the neurons of the irradiated spinal cord segment. This may serve as a physiological basis of increased FDG accumulation demonstrated by our PET-measurements.

Moreover, axons become temperature sensitive due to the sodium channel's response to higher temperature (Rasminsky 1972). When a nerve impulse triggers a node, essentially all sodium channels sequentially open and sodium enters the intracellular space, depolarizing the axon. But, the rate for sodium channels' closure is much more temperature dependent. Therefore, with an increase in temperature, they close faster decreasing the time allowed for current flow, which decreases total current production, and is another cause of conduction failure. Cooling has the opposite effect, increasing the time that channels are open, which improves efficient current production and prolongs continuous conduction. Studies of experimentally demyelinated axons show temperature sensitivity, such that a rise in temperature of as little as 0.5°C above normal will cause conduction failure in some demyelinated axons (Rasminsky 1972).

5. Conclusions

We can summarize the results of our investigations as follows. Radiation myelopathy usually runs a chronic, progressive, irreversible and relentless clinical course, with only rare recovery from established motor sequelae. The cases we have reported on, that represent at least partially reversible radiation injury, have common features from the point of view of tissue metabolism detectable with PET. However,

with the help of the other investigational methods applied, different (patho-) physiological processes could be identified in the background of these phenomena.

All patients exhibited increased FDG accumulation indicating elevated levels of glucose metabolism in those spinal cord segments that had been included in the radiation fields. Regional tissue perfusion measurements with $[^{15}O]$butanol showed parallel results, excluding severe deterioration of microcirculation. The increased tracer uptake observed can properly be attributed to a higher energy demand, since glucose is the primary fuel to nervous tissue. The need of this extra energy could not be attributed to cell proliferation on the basis of either the anamnestic data or the pathologic findings, and was further supported by the results of $[^{11}C]$methionine PET measurements.

In the asymptomatic case (Patient 1) we were lucky to observe and follow a „physiological" reaction to subthreshold dose of irradiation that could comprise mild remyelination and most likely only subtle inflammatory changes, i.e., vasculitis. The gradually diminishing FDG accumulation indicated the temporary nature of the changes. However, a different mechanism has to be presumed to be active in those cases with permanently increased glucose-metabolism in the affected regions (Patient 2, 3 and 4). The restoration of axonal conduction, reflected by the improvement of the clinical symptoms, suggests that an alternative conduction mechanism (continuous impulse propagation) takes place in order to overcome the blockade caused by the loss of myelin sheath. This implies an elevated number of Na^+-channels resulting in more effective Na^+/K^+-pump function, hence higher ATP demand.

In the case of LMND (Patient 5), the direct pathogenetic role of irradiation might be debated considering the lack of dose-dependence that is abundantly reported in the literature. Yet, a number of findings indicate the presence of a preceding viral infection in these radiogenic LMND cases, which may play a part in the evolution of the

symptoms (Berlit and Schwechheimer 1987; Bowen et al. 1996; de Carolis et al. 1986; de Greve et al. 1984; Feistner et al. 1989; Sadowsky et al. 1976; Tan and Pye 1991). Reactivation of the virus due to temporary immune deficiency caused by the radiation may elicit a persistent inflammation around the ganglion cells in the ventral horn. This low intensity reaction and the accompanying demyelination may lead to elevated glucose consumption.

There are no data available about PET-findings in patients with irreversible spinal cord injury, probably because of the lack of clinical relevance and their poor prognosis.

As a summary, we can conclude that PET investigation of radiation induced myelopathies of different severity provides new insights into the functional recovery of the cord suggesting mechanisms for the restoration of conduction not perceived so far.

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8. Appendix

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