**Materials and Methods:** we evaluated the protective effect of MnSOD and Cu/ZnSOD following 15Gy irradiation (IR) of the head and neck in male Wistar rats.

**Results:** SOD treatment did not protect the animals against irradiation induced reduction in oral intake and weight loss as well as against submandibular hypofunction. In contrast, MnSOD had a protective effect against irradiation induced hypofunction of the parotid gland. The mean parotid saliva flow rates were 84.1 6.5 ml/30 min in the controls, 22.2 4.3 ml/min following irradiation, and 38.5 4.5 ml/30 min following MnSOD therapy, respectively (n = 10) (P°0.05). Both MnSOD and Cu/ZnSOD demonstrated a protective effect against irradiation induced WBC suppression as the WBC of the controls was 15.5 × $10^9$/L; 6 days post (IR), the counts fell to 3.2 × $10^9$/L (n = 10) (P°0.01)., while following MnSOD or Cu/ZnSOD therapy, the WBC were 7.5 × $10^9$/L and 6.3 × $10^9$/L respectively (n = 10) (P°0.01).

**Conclusion:** these results indicate that SOD partially protects against head and neck irradiation induced injury while MnSOD may be superior to Cu/ZnSOD.

109 POSTER

## Effect of hypoxia on tumour growth and metastasis formation studied in chick embryo

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**Purpose:** Tissue oxygen supply influences the proliferation kinetics and vascularization of tumours. We studied the effect of hypoxia on tumour growth and metastasis formation in the early chick embryo.

**Materials and Methods:** For this study fertilised were incubated in an upright position in a commercial incubator at 36.8 ± 0.1°C and 60–65% relative humidity. The daily weight loss of the eggs was 0.3 ± 0.05 g per day which was considered to be within the normal range. The eggs were either incubated in air (20.9% oxygen, normoxia) or in 5% oxygen (hypoxia). After 48 hr approximately 4 × 4 mm of the inner egg shell were removed. In this area 4 × $10^5$ glioblastoma cells (human, U-138 MG) were implanted. After implanting the tumour cells the eggs were investigated by videomicroscopy. At the end of the experiment tissue specimen were taken and analysed for proliferating cell nuclear antigen (PCNA).

**Results:** Tumour growth was accelerated in hypoxia compared to normoxia. Furthermore, the shape of the tumour was different under these conditions. While during normoxia the growing tumours had a relatively sharp border, during hypoxia this was not the case. Metastasis were found only under hypoxic conditions. The determination of PCNA also showed clear differences between normoxic and hypoxic tumours.

**Conclusions:** Hypoxia itself obviously induces tumour cell proliferation. Whether or not this is due to autocrine or paracrine stimulation cannot yet be answered. Furthermore, hypoxia favours the formation of metastasis.

110 PUBLICATION

## Continous in vivo measurement of local mitochondrial metabolism after radiation

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**Purpose:** Different *in vitro* tetrazolium/formazan assays to determine radiation or drug effects on cell growth have been previously described. The aim of our study was to evaluate a new *in vivo* assay for continous measurements of local mitochondrial metabolism and cell proliferation.

**Methods:** For this study fertilized eggs were used. Irradiation of the area vasculosa (A.V.) was performed with a linear accelerator on day two of incubation. The eggs received doses from 2 to 10 Gy. For investigations of the local mitochondrial metabolism of the A.V. a micro-light guide spectrophotometer with modified light guides was used to measure the cleavage of a tetrazolium salt. 50 $\mu$m of the reagent (WST-1) were filled into the tip of the light guide. The WST-1 diffuses into the tissue and is cleaved to formazan. This is accompanied by a change in the absorption, which is measured as a calorimetric assay. In vitro calibration was performed with irradiated and non-irradiated single cell suspensions.

**Results:** There was a significant increase in absorption (optical density) measured by the calorimetric assay 1 hour after radiation with 10 Gy. Within 48 hours a significant decrease was observed compared to controls. After radiation with doses <10 Gy there was also a distinct, significant increase in absorption 1 h after irradiation. But then, optical density came to a normal range compared to the age matched control eggs.

**Conclusion:** The results show that it is possible to determine local metabolic activity and cell proliferation after irradiation continuously *in vivo*.

111 PUBLICATION

## Effect of ionizing radiation on cell-cycle progression as an expression of intrinsic radiosensitivity

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**Background:** The clinical outcome of radiotherapy is associated with the differences in the intrinsic radiosensitivity of human tumours. The amount of initial DNA double strand breaks is directly related to cellular radiosensitivity DNase when added fixes the damage and therefore modifies the pattern of progression through the cell cycle.

**Purpose:** To assess the *in vitro* intrinsic radiosensitivity in two cell lines derived from human tumours by flow cytometry after DNase was employed to fix the initial DNA damage induced by ionizing radiation (XRT).

**Methods:** Two cells line A549 (lung carcinoma) and A375 (melanoma) with different radiosensitivity $SF2_{A549}$ = 0.85 and $SF2_{A375}$ = 0.75 were irradiated at doses ranging of 2 Gy and incubated with various concentration of DNase over a wide range of time. After the enzyme was removed the cells stained with propidium iodide were analysed for DNA content by flow cytometry.

**Results:** There is a difference in the redistribution of cell lines XRT dose dependent. Whereas the more radioresistant A549 shows little and transient alteration of DNA content at low doses. A375 cell line exhibits a delay of progression through the cycle and an accumulation at the interfaces G1/S and S/G2. These alterations may be related to a greater inhibition of cyclins which control the cell cycle check-pints.

**Conclusion:** The method might be used as a simple and quick test to approximate radiosensitivity in limited tumour samples

112 PUBLICATION

## Radioperoxidation of human low-density lipoproteins (LDL): Antioxidant activity of a vitamin E derivative (MOH) -effect of the dose rate

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**Purpose:** The possible role of fatty acid peroxidation by free radicals is suggested by many epidemiologic and laboratory studies. We have evaluated the antioxidant effect of MOH and the effects of the dose rate on the peroxidation of LDL by free radicals produced by gamma radiolysis.

**Methods:** The effects of increasing doses have been followed by measuring the evolutions of the concentrations of endogenous vitamin E and "thiobarbituric acid-reactive substances" (TBARS).

**Results:** When MOH is in the solution, the consumption of endogenous vitamin E and TBARS are delayed, a higher dose is necessary to decrease the concentration of these compounds. Further more, the increase of the dose rate products a decrease of the yields of the two measured parameters of oxidation of LDL.

**Conclusion:** MOH exhibits antioxidant properties and prevents LDL against peroxidation by free radicals ($RO_2°/O_2°^-$) produced by gamma radiolysis. It is well know that these produced radicals are low initiators of lipid peroxidation. We can admit that these radicals react together in a preferential way. These reactions compete with the reactions of oxidation of LDL. The prevalence of the reactions of recombinaison leads to a decrease of the consumption of vitamin E and the formation of TBARS.