Summary

The many studies undertaken recently have increased the possibilities to use hyperthermia in cancer treatment. Heat treatment of tumours, which is usually at temperatures between 40 and 46°C, has two effects which are advantageous for cure. Cells appear to lose their clonogenic ability by the heat treatment itself (hyperthermic cell killing). Moreover, the cells surviving the heat treatment show an enhanced cell killing, when treated with radiation (hyperthermic radiosensitization). Both effects usually increase with treatment time and temperature. The combined heat and radiation treatment especially offers new potentials in cancer therapy. Not only in those cases where low radiation doses are prescribed, but as well because often radioresistant tumour cells will be hypersensitive to heat (see 1.1. and 8.7.).

Both from a scientific point of view, as well as in order to optimize the application of hyperthermia, it is important to know the mechanisms underlying hyperthermic cell kililng and radiosensitization. As radiation-induced cell death is probably caused by unrepaired DNA damage (see 1.2.), the experimental work described in this thesis is directed towards the effect of hyperthermia on DNA repair. Suspension cultured murine Ehrlich Ascites Tumour cells (EAT) and HeLa S_3 human cervical carcinoma cells were used for the experiments. The results were more or less identical for both cell lines.

Hyperthermia inhibits the rate of repair of DNA strand breaks, an ubiquitously occurring type of DNA damage after radiation (chapter 2) and reduces the activity of DNA polymerases, which are known to be involved in DNA repair (chapter 5). Modification of the heat treatment was used as a tool to study the relationship between strand break repair and DNA polymerase activity on one hand, and hyperthermic cell killing and radiosensitization on the other. The cell-killing effect of hyperthermia can be reduced by the addition of erythritol during the heat treatment or by the induction of thermotolerance by repeated heat treatments. Cell killing can be enhanced by addition of procaine during hyperthermia or by "step-down" heating (thermosensitization). The extent of hyperthermic cell killing, radiosensitization, inhibition of DNA polymerase activity, and the rate of strand-break repair were compared under the different circumstances.

The hyperthermic inhibition of **strand-break repair** was influenced qualitatively as well as quantitatively by procaine, erythritol (chapter 4) and thermotolerance (chapter 6) in the same way as observed for hyperthermic cell killing.

A small number of **DNA strand breaks** was observed to arise from hyperthermia alone (chapter 3). As the number of these strand breaks was not influenced by the presence of procaine, erythritol or thermotolerance, this type of DNA lesion could not directly be related to hyperthermic cell killing. These strand breaks are more probably caused by alterations in the nuclear skeleton (see below), for example as a result of torsion of the adhered chromatin. Further investigations into the formation and repair of these breaks after lower heat doses and their cell cycle dependency are necessary before definite conclusions can be drawn (chapter 8).

DNA polymerase activity (especially polymerase β activity) was influenced in the same was as cell killing by the presence of procaine or erythritol during the heat treatment (chapter 5). These agents act indirectly, probably at the level of the plasma membrane, as the effects were only observed in the case of intact cells and not when

isolated nuclei were used (chapter 5). The lack of an effect of thermotolerance or thermosensitization at the level of hyperthermic DNA polymerase inactivation (chapter 5,6,7), shows that the inhibition of polymerase activity can neither explain hyperthermic cell killing nor the hyperthermic inhibition of strand-break repair.

Likewise, the extent of hyperthermic **radiosensitization** was uninfluenced by thermotolerance or thermosensitization (chapter 6,7). Therefore, hyperthermic radiosensitization appears to be unrelated to the inhibition of strand-break repair, which was influenced by thermotolerance. A high correlation coefficient was found for the relationship between radiosensitivity and the extent of polymerase inactivation in thermotolerant cells (chapter 6). After thermosensitization this value was lower than expected, however, which implicates that the reduced polymerase activity cannot fully explain hyperthermic radiosensitization either.

Alterations in **nuclear structure** after hyperthermia are expressed as an enhanced protein content and a reduced sedimentation of nuclei isolated from heated cells (chapter 5). Other reports in the literature indicate that these alterations are attended with a reduced accessibility of the DNA for repair enzymes.

In conclusion the results show that DNA repair is inhibited by hyperthermia through membrane damage, inactivation of repair enzymes, as well as alterations in chromatin structure. The inhibition of the DNA repair capacity is the most likely cause of hyperthermic radiosensitization. The parameters investigated, strand-break repair capacity and DNA polymerase activity, do not appear to be the rate limiting step under all circumstances. Above all, the alteration in chromatin structure has to be investigated into more detail. A reduced DNA repair capacity of naturally occurring or hyperthermia-induced DNA damage, may as well be the cause of cell killing by heat alone.