Abstract. Experiments in vitro have shown that dehydroascorbic acid (DHA) possesses antitumor properties under irradiation, which are gradually enhanced by combination with β-carotene or vitamin E. On the other hand the cytostatic efficiency of mitomycin C (MMC) is increased from $D_{37} = -93$ up to $D_{37} = -141$ in the presence of DHA. It has also been shown that Escherichia coli bacteria are able, to some extent, to reduce DHA to ascorbate under the same experimental conditions. The results are of interest for the radiation therapy of cancer.

It has been previously demonstrated that the efficiency of cytostatic agents, e.g. mitomycin C (MMC), is strongly enhanced in the presence of antioxidant vitamins (C, E and β-carotene) under irradiation in the presence of air (1-4, 7). These findings are based on experiments in vitro using bacteria and/or cancer cells and on pulse radiolysis studies of these substances. Thereby the activation of MMC to its biologically active, semiquinone form (MMC*) is ensued by a cascade electron transfer with the sequence: $\text{AH} \rightarrow \text{vit.E}^{*+}/\text{vit. E} \rightarrow \beta\text{-car}^{*+}/\beta\text{-car} \rightarrow \text{MMC/MMC}^{*}$ in the cell (3). In the first step of this process, the resulting vitamin C (ascorbate, $\text{AH}^{*}$) radical disappears by forming ascorbate (AH-) and dehydroascorbate (DHA) (5-7). A free radical interaction between the vitamins E and C has also been reported (8).

On the other hand, it has been established that extracellular dehydroascorbate (DHA) can be reduced to ascorbate (AH-) when incubated with K562 erythroleukaemic cells (9). The process can be stimulated by glucose and inhibited by e.g. phloretin and N-ethylmaleimide. It is dependent on temperature, the number of K562 cells and on the DNA concentration, but it is not proportional to time. It has been further demonstrated that ascorbate regeneration by DHA reduction is accomplished by membrane-bound ascorbate free radical reductase (semihydro-ascorbate-reductase) and enzymatic or non-enzymatic reduction of DHA in various biological systems (10-12).

Based on the findings mentioned above, it was of interest to study the specific effect of DHA, as well as of its mixture with vitamin E and β-carotene, on MMC efficiency under γ-irradiation. MMC was used as a representative cytostatic agent and Escherichia coli bacteria (AB 1157) as a model of living systems.

Materials and Methods

All used chemicals were of p.a. purity and the solutions were prepared with 4 times distilled water. The irradiation source “Gammacell 220” (Nordion Intern. Inc., Canada) provided a dose rate (DR) of 75 Gy/min. The handling procedure for the bacteria as well as the evaluation of the results have been previously reported (13,14). The bacteria were added to the buffer and, after one-hour incubation, they were irradiated with γ-rays. In the other series of experiments, the individual components or mixtures of them were added to the buffer as indicated in Figure 1 and Table I, respectively. The survival curves show the N/No-ratio (No = number of bacteria colonies before and N = after irradiation) as a function of absorbed radiation dose (Gy). The $D_{37}$-value represents the dose (Gy) at N/No = 0.37 of each survival curve, whereas the $\Delta D_{37}$-values (Gy) are calculated by subtracting the $D_{37}$-buffer from the individual $D_{37}$-data, e.g. $D_{37}$ (sample)-$D_{37}$ (buffer) = $\Delta D_{37}$ (sample). The positive $\Delta D_{37}$-values indicate the radiation protecting property of a given system, whereas the negative ones show its cytostatic efficiency. The high purity vitamins were provided by Hoffmann-La Roche Corp. (Basel, Switzerland). MMC (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) was used as obtained (ampoule of 2 mg MMC mixed with 48 mg NaCl).
Results and Discussion

By irradiation of biological systems in aqueous media, the primary products of water radiolysis can initiate various processes. The water radiolysis is expressed by gross reaction (1) and the yields of the radiolytic products (G-values = number of molecules formed per 100 eV absorbed energy) are given in brackets for the pH-range of 6 - 8.5:

\[
\text{H}_2\text{O} \rightarrow e_{\text{aq}}^-, \text{H}^+, \text{OH}^-, \text{H}_2, \text{H}_2\text{O}_2
\] (1)

In the presence of air, the "solvated electrons" (e_{aq}^-) and the H-atoms are converted into peroxy radicals by reaction with oxygen (15):

\[
\begin{align*}
\text{H}^+ + \text{O}_2 & \rightarrow \text{HO}_2^- \\
e_{\text{aq}}^- + \text{O}_2 & \rightarrow \text{O}_2^- \\
\text{HO}_2^- & \rightarrow \text{H}^+ + \text{O}_2^-
\end{align*}
\] (2) (3) (4)

The experiments in the present study were performed in aerated aqueous media at pH = 7.4. Therefore, under these conditions 46% OH^* radicals and 54% O_2^- species initiate the observed processes.

In accordance with the strong synergistic effect of the antioxidant vitamins (C, E and β-carotene) on the efficiency of cytostatic agents, it was of interest to investigate the effect of DHA in this respect. The change of the N/No-ratio as a function of absorbed radiation dose was studied for MMC and DHA as well as for mixtures of both in the presence of individual antioxidant vitamins. The obtained results are presented in Figure 1.

It is evident that all investigated systems exhibit cytostatic properties. The corresponding ΔD_{37}-data are compiled in Table I. Whereas the ΔD_{37} values of DHA and of DHA in the presence of β-carotene or vitamin E are lower than that of MMC, the combination of MMC with DHA and β-carotene and with DHA and vitamin E or vitamin C as well as the mixture of DHA, vitamin E, β-carotene and MMC...
show a gradual increase of the -ΔD37-values. The highest one was, however, observed for the mixture of DHA and MMC, having ΔD37 = -141. It is 50% higher than the value of MMC alone, ΔD37 = -93 (see Figure 1 and Table I). The effect of vitamin E and β-carotene in this respect appears to be very similar to that previously observed for mixtures of these two vitamins with MMC (2,7).

On the other hand, the question arises as to whether the used E. coli bacteria can reduce DHA to ascorbate, similarly as already mentioned for K562 erythroleukaemic cells (9). The results of some preliminary experiments in this respect are shown in Figure 2.

In this case, the starting media contained 1x10^{-4} mol/L DHA in phosphate buffer (pH = 7.4) and *Escherichia coli* bacteria (AB 1157) in similar concentration as in the experiments presented in Figure 1. The transformation of DHA to ascorbate was registered at 20±1°C (A) and 38±1°C (B) as a function of incubation time (hours) by measuring the absorption at 261 nm. For 1h incubation at 20°C about 9% (9x10^{-6} mol/L) and at 38°C more than 20% (2x10^{-5} mol/L) ascorbate was formed. The maximum values were observed after about 7 h incubation time. This fact demonstrates that, in the media containing DHA and MMC (Figure 1, ΔD37 = -141), about 80% of the observed effect can be attributed to the interaction of DHA and about 20% to ascorbate. As can be seen in Figure 1 and Table I, the mixture of 10^{-4} mol/L ascorbate and 10^{-5} mol/L MMC results in a ΔD37 = -112.

### Conclusion

The comparison of ΔD37-values obtained from experiments using DHA, DHA in the presence of vitamin E or β-carotene, as well as of MMC in mixtures with vitamin C, DHA and vitamins (Table I) demonstrate that DHA can contribute to an essential increase of the MMC efficiency.

### Acknowledgements

The authors are indepted to Greiner Holding GmbH, KG & AG, Kremsmünster, Austria for providing radiation-sterilized laboratory utensils as well as to Hoffmann –La Roche (Vitamin & Fine Chemical Division) Basel, Switzerland for providing the vitamins.

### References