Scanning Microscopy

Volume 1 | Number 4 Article 33

7-17-1987

# Effects of Cis-Dichlorodiammineplatinum Alone and in Combination with Ionizing Radiation on the Esophageal Mucosa: A Scanning and Transmission Electron Microscopic Study

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Scanning Microscopy, Vol. 1, No. 4, 1987 (Pages 1861-1869) 0891-7035/87$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

# EFFECTS OF CIS-DICHLORODIAMMINEPLATINUM ALONE AND IN COMBINATION WITH IONIZING RADIATION ON THE ESOPHAGEAL MUCOSA: A SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY

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(Received for publication February 5, 1987, and in revised form July 17, 1987)

## Abstract

Cis-dichlorodiammineplatinum (cis-DDP) has for more than 20 years been part of the therapeutic arsenal of oncology. Most of the knowledge about its biological action is based on clinical investigations and therefore an examination of the influence of cis-DDP at the cellular and sub-cellular level is necessary. Five mg of cis-DDP was given intraperitoneally (i.p.) to ten rabbits. Ultrastructural examinations were performed on the upper and lower parts of the esophagus each day after the injection on the following ten days. Another 50 rabbits were given 5 mg cis-DDP and were irradiated in an area just beneath the hypopharynx. They were given 2 Gy at each irradiation and were maximally treated with up to 20 Gy. Examinations were carried out from the first day after the final treatment and each day during ten consecutive days. Five animals were used as controls. Cis-DDP proved to have a deleterious effect on the epithelial layer of the esophageal mucosa with cell loss and structural disarrangement of the microridges and whorls on the surface. This finding was an early phenomenon and lasted for all ten examination days. The changes were not more exaggerated when irradiation was added to the experiments. Repopulation of new cells from the matrix was noticed about five days after the administration of cis-DDP alone.

Key Words: Esophagus, epithelium, cis - dichlorodiammineplatinum (cis - DDP), fractionated irradiation, scanning electron microscopy, transmission electron microscopy.

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## Introduction

Cis-diamminedichloride (cis-DDP, cis-Platinum, cis-P) has since 1971 been used as an antitumor chemotherapeutic drug, based on the findings of Rosenberg et al. (1965, 1969), Howle and Gale (1970) and Rosenberg (1971). Since then it has asserted its raison d'etre among the highly potent cytostatic drugs. Clinically it exerts its greatest action on the testicular tumors (Williams and Einhorn, 1980), head and neck cancer (Jacobs, 1980) and ovarian carcinoma (Holland et al., 1980) but it is also used in the treatment of many other tumors. Among these, esophageal carcinoma is one target.

The biological action of cis-Platinum is multifactorial. It has a cytotoxic effect which, acting on DNA, is in many ways equivalent to the action of alkylating agents (Pascoe and Roberts, 1974, Butour and Macquet, 1977). This effect is tumoricidal, but also dose-limiting especially because of the nephrotoxic effects of the drug (Rossof et al., 1972, Madias and Harrington, 1978, Comis 1980).

Cis-platinum has also a synergistic effect with ionizing radiation since it has a high electron-affinity (Richmond and Simic, 1978) and a substantial power to inhibit repair of radiation induced damage (Douple and Richmond, 1978).

Clinical reports on the early toxicity of combined treatment are sparse and seldom contain detailed information: this makes it important to examine the effects of such a modality of therapy.

So far no ultrastructural research work regarding the effects of concomitant radiation and chemotherapy on the esophagus has been reported. The acute reaction to chemotherapy of this normal tissue is a limiting factor in the treatment of patients with malignant tumors in the head and neck region, pulmonary and esophageal carcinoma. Therefore, this paper deals with an experimental study of the effect of cis-platinum and irradiation on the normal tissue of an animal (esophagus in rabbits). Light microscopy (LM), scanning and transmission electron microscopy (SEM and TEM) were used. We have attempted to classify and clarify very early effects. Repair and repopulation were observed in this rapidly proliferating tissue. The results obtained appear to have direct clinical implications.

## Materials and Methods

The material selected for this study consisted of 65 full-grown rabbits weighing 1.8 - 2.3 kg. Five

animals acted as control animals. Fifty rabbits received fractionated irradiation according to schedule, (Fig. 1). The rabbits were treated with cis-DDP before the first irradiation treatment in each dose group. The effect of irradiation alone on the esophagus of the rabbit is described in Albertsson et al. (1987). For the evaluation of cis-DDP relevant data have been extracted from that work.

Irradiation

Each rabbit was anaesthetized for about 15 minutes during the administration of irradiation by intraperitoneal injection of pentobarbital, (40 mg per kg body weight). Irradiation was delivered by a Siemens X-ray machine operating at 160 kV X-ray, filtered by 4 mm Al, at a focus skin distance of 50 cm, giving an absorbed dose of 2 Gy to 2 cm of the esophagus just beneath the hypopharynx. The absorbed dose in the esophagus was controlled by thermoluminescent dosimeters. Fifteen mm beyond the caudal part of the irradiated area the absorbed dose was negligible. The distance between the irradiated area and control area was 40 mm.

Drug

Cis-diamminedichloroplatinum (II), (cis-DDP, cisplatinum), (Platinol$^R$, Bristol Myers Company) was available in 20 ml flasks dissolved in normal saline at a concentration of 0.5 mg/ml.

Experiments

The rabbits were treated with fractionated irradiation, (2 Gy/F), with a total dose ranging from 2-20 Gy. About 30 minutes before the first irradiation treatment in each dose group, each animal was injected with 5 mg cis-DDP intraperitoneally according to the schedule in Fig. 1. The rabbits were laid on their backs and the upper part of the esophagus was irradiated. The animals were treated in groups of ten. After completion of irradiation one animal was taken out from the groups on ten consecutive days. The animals were sacrificed by a blow on the skull in order to avoid pharmacological side-effects. The esophagus was dissected out in its entire length (9 cm). Samples for SEM, TEM and LM were taken from the upper part of the esophagus (irradiated area: $E'_x$) and the lower part of the esophagus (control area: $E'_0$). Control investigations were also performed in the same way on untreated animals.

Preparation for SEM

The pieces for SEM-examination were not rinsed, but were placed directly in 2.5 glutaraldehyde for fixation for 12 h, (in 0.15 M cacodylate buffer). The pH of the solution was 7.3. They were then transferred into the same buffer, and were later osmium-fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for 2 h. Dehydration was carried out with a graded series of ethanol, after which the preparations were transferred to Freon TF 618. The specimens were later dried according to the critical point method in a Balzer - 000 critical point dryer. They were finally sputter-coated with gold plus palladium in a Polaron coating unit E 5000. Then they were examined in a Cambridge Stereoscan Mark II A or a Zeiss Nanolab electron microscope. The microscopes were operated at 20 kV.

Preparation for TEM

The samples were fixed as for the SEM-preparations, and additionally in 1% osmium tetroxide in 0.15 M cacodylate buffer (pH = 7.3) for 2 h, rinsed in 0.15 M cacodylate buffer, dehydrated in ethanol and embedded in Vestopal W or Epon. Secretions of 1 µm thickness were cut on an LKB-ultrotome, stained with toluidine blue and examined in a light microscope. Ultrathin sections were cut and contrasted with lead citrate and uranyl acetate or en bloc with 0.5 uranyl acetate. A Zeiss EM 10 electron microscope was used for examination.

Bacterial control

Cultivation of bacteria from the upper and lower end of the esophagus was performed in 8 animals.

pH-measurements

This was made at both the upper and lower end of the esophagus.

Statistical analyses

A four-way analysis of variance with repeated measurements on one factor was used.

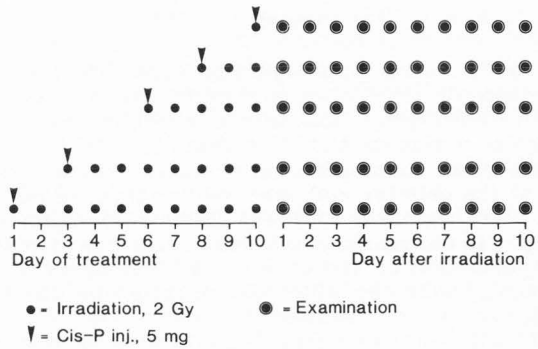



**Figure 1.** Treatment schedule for combined cis-DDP and irradiation. The drug was given at a dose of 5 mg about 30 minutes before the first irradiation. Total dose ranged from 5 mg cis-DDP / 2 Gy to 5 mg cis-DDP/20 Gy. After completion of treatment, experiments were made daily from day 1 to day 10.

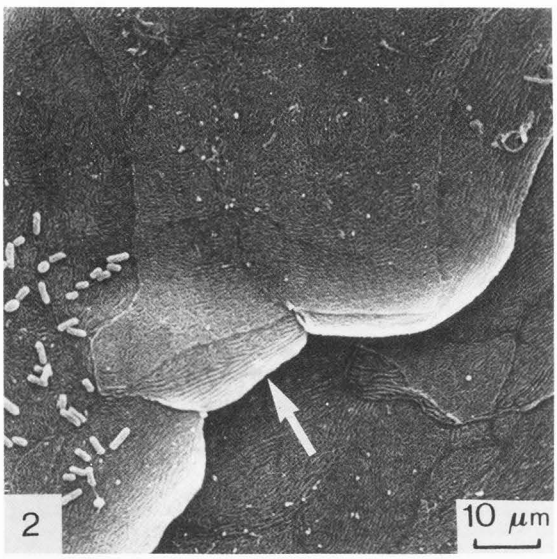
## Results

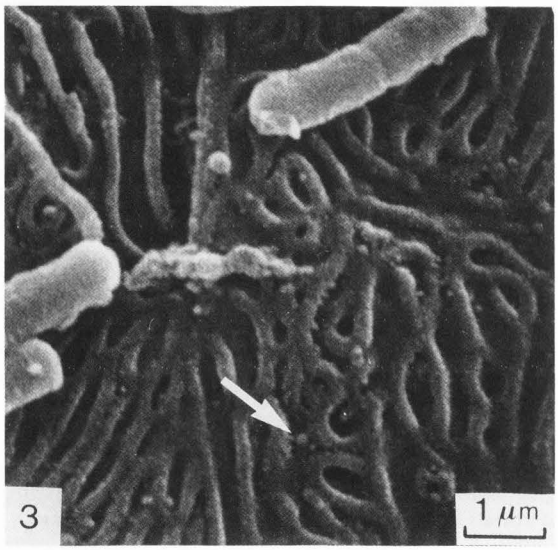
Control material

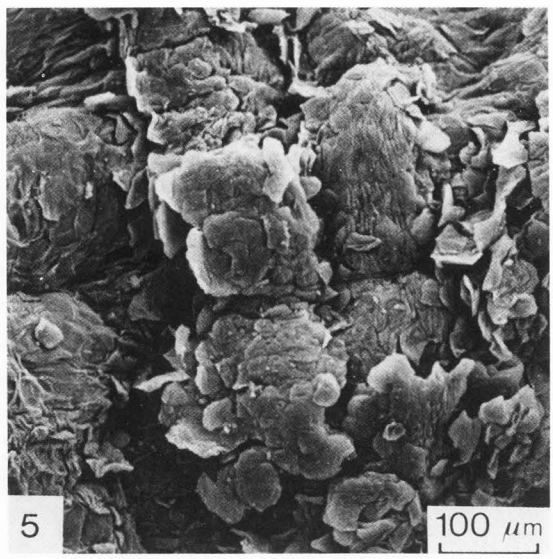
Both the upper part of the esophagus close to the hypopharynx $E_x$ and the lower part of the esophagus $E_0$ were examined. No difference in the ultrastructural pattern was observed between these two different parts. In the five untreated rabbits the ultrastructure consistently presented a homogeneous pattern. SEM showed polygonal regularly arranged cells attached to each other with discrete cell lines. Cell loss occurred as a natural phenomenon with loss of flakes composing groups of cells (Fig. 2). On the surface numerous microridges were seen (Fig. 3).

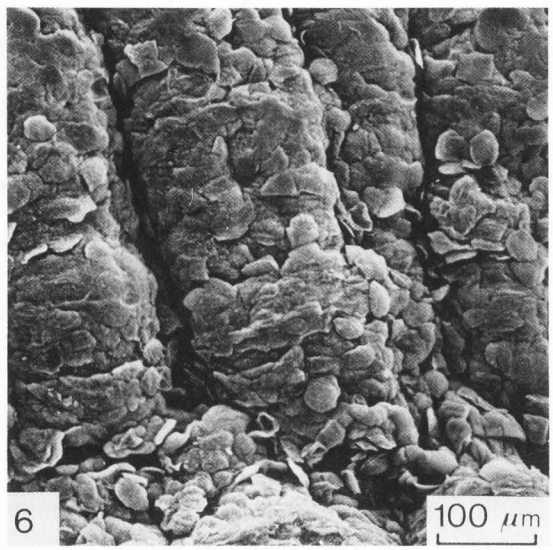
A variable amount of bacteria was observed on the esophageal surface, cocci and rods. Cultivation of the bacteria showed mostly E. coli. However, occasionally B. catarrhalis, Acinetobacter, Bacillus sp. and Haemophilus sp. were found. Also a small amount of detritus and desquamation products could be seen by SEM on the surface.

TEM showed a basal layer of columnar cells, a prickle cell layer: several layers of polygonally shaped cells with numerous intercellular bridges and finally a functional cell layer with several layers of cells, increasingly flattened towards the lumen with their long axes parallel to the surface and with pyknotic nuclei, cf. Hopewood et al. (1977).

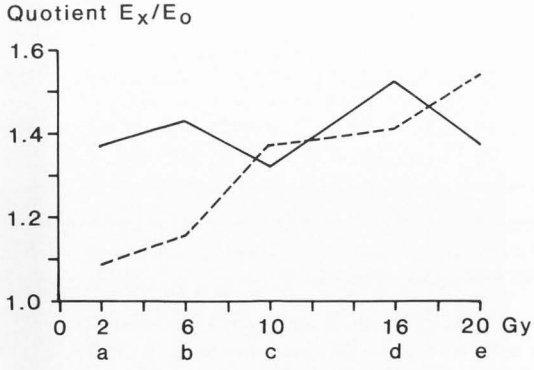



**Figures 2 and 3.** SEM-micrograph of normal esophageal mucosa. **Figure 2.** Cell loss is observable (arrow). **Figure 3.** Regularly arranged microridges are seen, a small amount of bacteria and occasionally small knoblike structures on the microridges (arrow).




**Figures 5, 6 (above).** SEM-micrographs illustrating the edema of the esophageal mucosa.
**Figure 4 (left).** The relationship between the height of the esophageal mucosa within irradiated area compared to the control area for each dose group a-e 1-10 days after irradiation fractionated irradiation cis-DDP + fractionated irradiation, (----fractionated irradiation, described in Albertsson et al. 1987).

Cis-DDP and irradiation

Edema. LM micrographs were taken of the upper ($\overline{E'_x}$) and lower ($E'_0$) parts of the esophagus. By measuring the thickness of the epithelium a difference was found between the upper irradiated area ($E'_x$) and the control area ($E'_0$). All preparations within the irradiated area showed an edema of the mucosal epithelium. This phenomenon was already noticeable within the low dose range (2 Gy) and throughout the whole series without any significant dose-dependency (Fig. 4). This is in contradiction with the edema observed in the fractionated radiation group where edema could also be calculated

Table 1. Estimation of the number of loosened microridges at different doses on the surface*

| Total dose | Day 1 - 5 | | | | Day 6 - 10 | | | |
|---|---|---|---|---|---|---|---|---|
| | Area | Smv | Area | Smv | Area | Smv | Area | Smv |
| 2 Gy | $E'_x$ | 0.6 | $E_x$ | 1.0 | $E'_x$ | 0.3 | $E_x$ | 0.8 |
| | $E'_o$ | 0.3 | $E_o$ | 0.3 | $E'_o$ | 0.1 | $E_o$ | 0.15 |
| 6 Gy | $E'_x$ | 0.4 | $E_x$ | 1.5 | $E'_x$ | 0.5 | $E_x$ | 1 |
| | $E'_o$ | 0.4 | $E_o$ | 0.05 | $E'_o$ | 0.35 | $E_o$ | 0.05 |
| 10 Gy | $E'_x$ | 1.45 | $E_x$ | 1.1 | $E'_x$ | 1.4 | $E_x$ | 0.5 |
| | $E'_o$ | 0.7 | $E_o$ | 0.15 | $E'_o$ | 0.95 | $E_o$ | 0.1 |
| 16 Gy | $E'_x$ | 1.9 | $E_x$ | 2.2 | $E'_x$ | 0.6 | $E_x$ | 0.5 |
| | $E'_o$ | 0.35 | $E_o$ | 0.15 | $E'_o$ | 0.2 | $E_o$ | 0.15 |
| 20 Gy | $E'_x$ | 1.5 | $E_x$ | 2.4 | $E'_x$ | 1.4 | $E_x$ | 0.9 |
| | $E'_o$ | 0.6 | $E_o$ | 0.2 | $E'_o$ | 0.5 | $E_o$ | 0.1 |

Smv = scored mean value *Data for $E_x$ and $E_o$ from Albertsson et al. (1987)
0 = 0 - 50; 1 ∿ 100; 2 ∿ 200; 3 ∿ 300
$E'_x$ = Cis-P + Fractionated irradiation $E'_o$ = Controls Cis-P
$E_x$ = Fractionated irradiation $E_o$ = Controls

Table 2. Estimations of the number of cell loss at different doses on the surface*

| Total dose | Day 1 - 10 | | | |
|---|---|---|---|---|
| | Area | Smv | Area | Smv |
| 2 Gy | $E'_x$ | 1.3 | $E_x$ | 0.8 |
| | $E'_o$ | 1.0 | $E_o$ | 0.6 |
| 6 Gy | $E'_x$ | 1.2 | $E_x$ | 1.1 |
| | $E'_o$ | 0.5 | $E_o$ | 0.6 |
| 10 Gy | $E'_x$ | 1.3 | $E_x$ | 1.5 |
| | $E'_o$ | 1.3 | $E_o$ | 0.7 |
| 16 Gy | $E'_x$ | 1.7 | $E_x$ | 1.6 |
| | $E'_o$ | 1.3 | $E_o$ | 0.9 |
| 20 Gy | $E'_x$ | 2.0 | $E_x$ | 1.6 |
| | $E'_o$ | 1.2 | $E_o$ | 0.7 |

*Data for $E_x$ and $E_o$ from Albertsson et al. (1987)
Smv = Scored mean value
0 < 50; 1 = 51-100; 2 = 101-150; 3 > 150
$E'_x$ = Cis-P + Fractionated irradiation
$E'_o$ = Control Cis-P
$E_x$ = Fractionated irradiation
$E_o$ = Controls

from LM-micrographs. However, within this series a significant dose-relationship was found (Fig. 4). Figs. 5 and 6 illustrate the edema of the esophageal mucosa on SEM-micrographs.

Loosening of microridges

SEM of the normal untreated esophagus showed microridges that had loosened from the surface and were protruding like small knobs or snakes on the surface (S.A.K.s). A scoring system considering dose and time and based on the number of the S.A.K.s as calculated from SEM pictures (5,000 X) was devised and is presented in Table 1. Figs. 7-9 illustrate S.A.K.s scored from 1-3. Treatment with fractionated irradiation caused a significant increase in the number of S.A.K.s during the first five days after completion of irradiation (Albertsson et al., 1987). The addition of cis-DDP seemed to cause quite a different pattern. The number of S.A.K.s increased within the irradiated part of the esophagus, however, were not significantly dose-related. This was valid for all ten days of observation. The esophageal part receiving the combined modality (cis-DDP + irradiation) had a surface with damaged microridges but to a lesser degree when compared with the specimens treated with irradiation alone (Table 1).

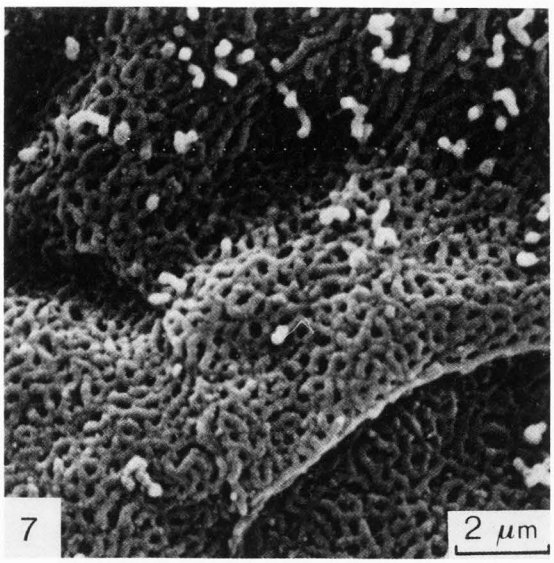
Within the lower part of the esophagus ($E'_0$) the number of damaged microridges was increased compared to the control animals and also compared to control preparations from the animals treated with irradiation in the upper part of the esophagus ($E_0$).

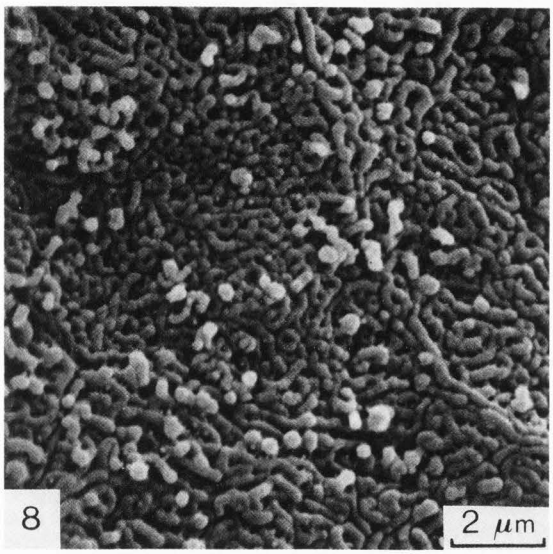
Cell loss

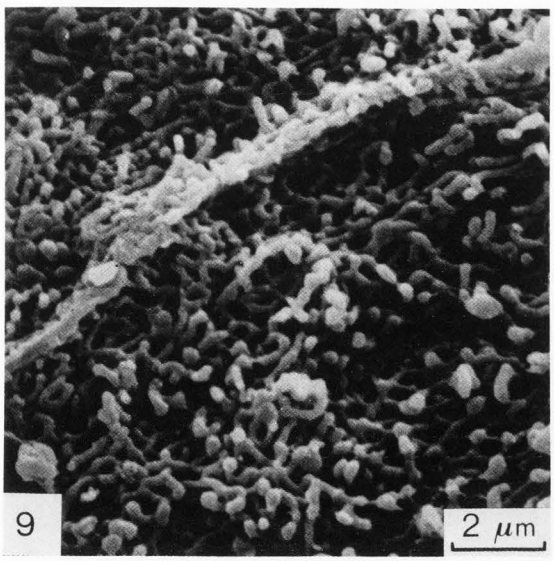
As a naturally occurring phenomenon cell loss is observed from the SEM surface in an amount of less than 100 on a defined area from a 100 X magnification SEM picture. This is interpreted as normal desquamation process, where the cells often seem to loosen in flakes composed of groups of several cells. A scoring system was made up (cf Albertsson et al. 1987), whereby the number of loosened cell flakes on a 100 X magnification SEM picture was counted and the results are presented in Table 2. Figs. 10-12 illustrate cell loss scored 1-3. Treatment with cis-DDP and fractionated irradiation resulted in an increase in cell loss both within the combined treatment area ($E'_x$) and the control area ($E'_0$) (Table 2).

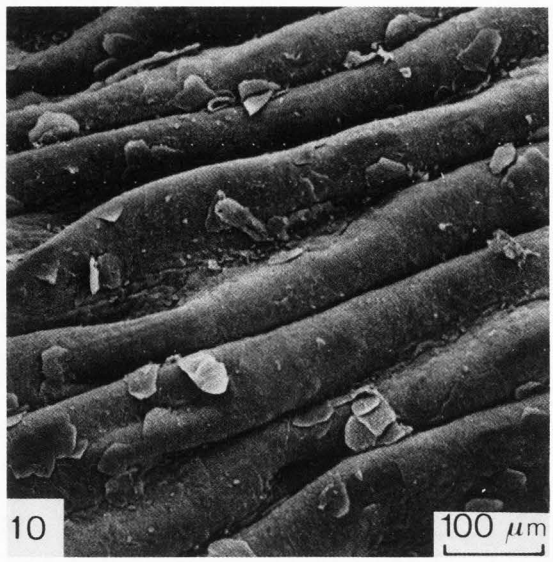
**Figures 7-9.**. SEM micrographs illustrating loosened microridges: score = 1 **(Fig.7)**; score = 2 **(Fig.8)**; and score = 3 **(Fig.9)**.

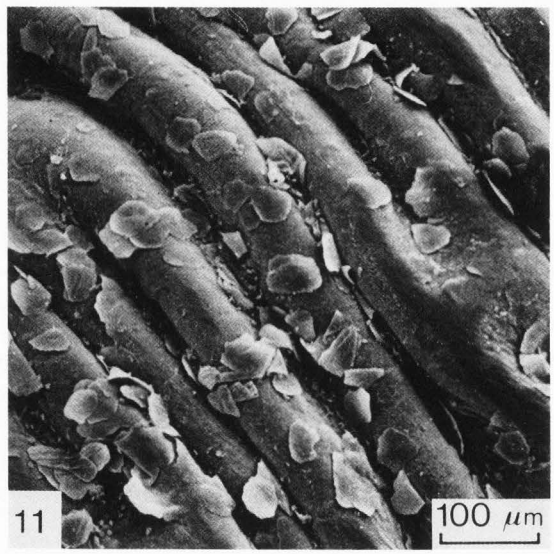
**Figures 10-12.** SEM micrographs illustrating cell loss: score = 1 **(Fig.10)**; score = 2 **(Fig.11)**; and score = 3 **(Fig.12)**.

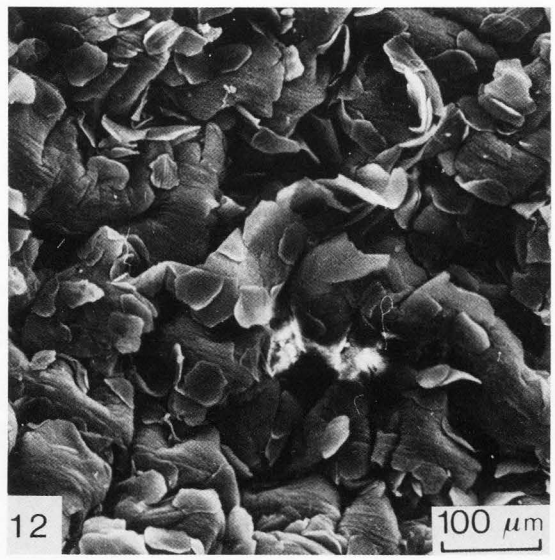

7
2 μm


8
2 μm

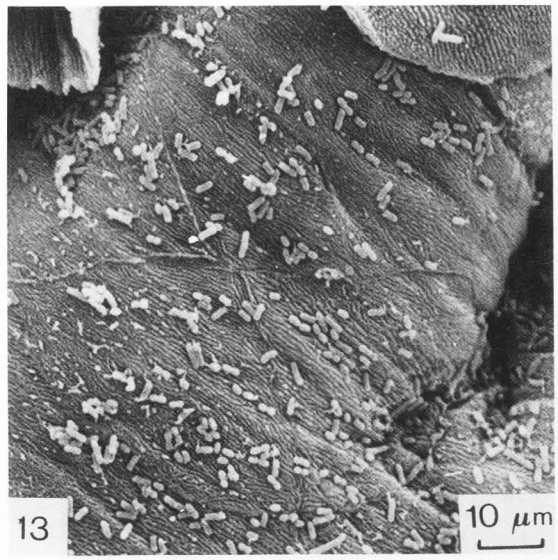

9
2 μm

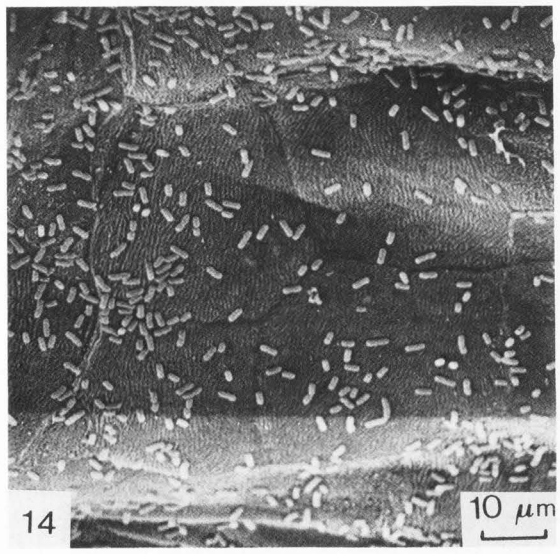

10
100 μm

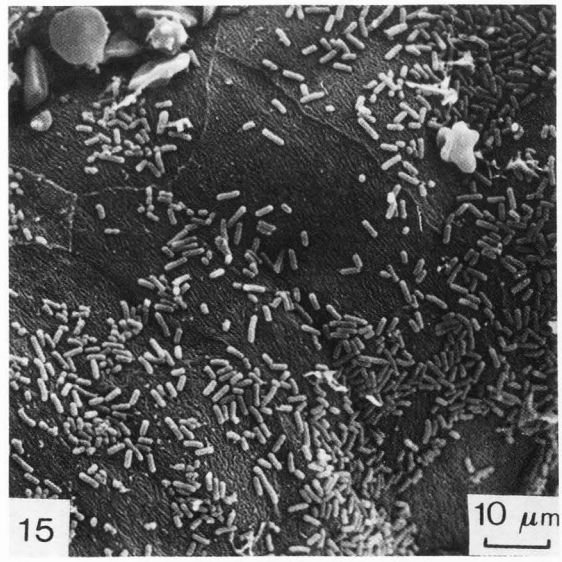

11
100 μm


12
100 μm

**Figures 13-15.** SEM micrographs illustrating bacteria: score = 1 **(Fig.13)**; score = 2 **(Fig.14)**; and score = 3 **(Fig.15)**.




No significant variation could be observed during the ten observation days. Therefore, all values were gathered in one point. Also for comparison, the cell loss within the fractionated irradiation group is presented in Table 2. Treatment with fractionated irradiation resulted in an increased cell loss within the irradiated area ($E_x$), however, not significantly dose related. Within the control parts ($E_0$) of the same specimens, the cell loss seemed rather constant and did not differ in that respect from the untreated control animal. However, the addition of cis-DDP seemed to cause an increase in cell loss both within the combined treatment area ($E'_x$) and the control area ($E'_0$). Compared to control animals, the number of loosened cell flakes was raised already after 5 mg cis-DDP, ($E'_0$), and 5 mg cis-DDP + 2 Gy ($E'_x$). In the group treated with cis-DDP alone, the cell loss was increased, but it remained almost at the same level for all doses and throughout the observation period. Within the combined treatment area ($E'_x$) the cell loss was slightly but not significantly positively dose-related and not significantly increased compared to the group treated with fractionated radiation alone ($E_x$).

Bacteria

The esophageal surface is covered with bacteria (Albertsson et al., 1987) in an amount of 100 - 200 on a defined area 17x11 cm from a 100 X magnification SEM picture. Estimations of the number of bacteria based on a scoring system were made and the results are presented in Table 3. Figs. 13-15 illustrate bacteria scored 1-3. After completion of radiation for all dose groups the amount of bacteria on the surface was reduced during the first half of the observation period. However, a recurrence of the bacteria was observed with a bacterial content above the normal value in some cases during days 6-10 after completion of treatment. This was valid both for the fractionated treatment group ($E_x$, $E_0$) and the combined treatment group ($E'_x$, $E'_0$). Moreover the control area ($E_0$, $E'_0$) and irradiated area ($E_x$, $E'_x$) seemed to be dependent on each other in this respect, since the occurrence of bacteria in the upper and lower part of the esophagus for each animal showed a common pattern.

## Discussion

The present research was designed to investigate the acute toxicity of cis-DDP on the esophagus using ultrastructural methods. Recent developments in radiotherapy point to more intensive treatment, whereby the early normal tissue tolerance is the dose-limiting factor. Our knowledge of the early effects of combined treatment (chemotherapy and radiation) on normal tissues, as well as of the effects on normal tissues of changed fractionation schedules is very limited. The combined modality (cis-DDP and irradiation) resulted in an edema of the rabbit esophageal mucosa, reaching a maximum already after 5 mg cis-DDP + 2 Gy and thereafter remaining fairly constant throughout the observation period, without any significant dose dependency. Treatment with fractionated radiation alone also results in an edema of the irradiated epithelium, which is positively dose-

Table 3. Estimation of the number of bacteria at different doses on the surface*

| Total dose | Day 1 - 5 | | | | Day 6 - 10 | | | |
|---|---|---|---|---|---|---|---|---|
| | Area | Smv | Area | Smv | Area | Smv | Area | Smv |
| 2 Gy | $E'_x$ | 1.3 | $E_x$ | 0.9 | $E'_x$ | 0.2 | $E_x$ | 1.3 |
| | $E'_o$ | 0.25 | $E_o$ | 1.0 | $E'_o$ | 0 | $E_o$ | 1.0 |
| 6 Gy | $E'_x$ | 0.25 | $E_x$ | 0.7 | $E'_x$ | 1.1 | $E_x$ | 1.1 |
| | $E'_o$ | 0.8 | $E_o$ | 1.1 | $E'_o$ | 1 | $E_o$ | 1.5 |
| 10 Gy | $E'_x$ | 0.4 | $E_x$ | 0.6 | $E'_x$ | 0.4 | $E_x$ | 2.1 |
| | $E'_o$ | 0.3 | $E_o$ | 0.7 | $E'_o$ | 1.7 | $E_o$ | 1.4 |
| 16 Gy | $E'_x$ | 0.6 | $E_x$ | 0.5 | $E'_x$ | 1.5 | $E_x$ | 0.6 |
| | $E'_o$ | 0.3 | $E_o$ | 0.5 | $E'_o$ | 0.4 | $E_o$ | 0.6 |
| 20 Gy | $E'_x$ | 0.95 | $E_x$ | 0.7 | $E'_x$ | 1.75 | $E_x$ | 0.1 |
| | $E'_o$ | 0.7 | $E_o$ | 0.6 | $E'_o$ | 0.85 | $E_o$ | 0.1 |

Smv = scored mean value *Data for $E_x$ and $E_o$ from Albertsson et al. (1987)
0 = 0 - 25; 1 ∿ 200; 2 ∿ 400; 3 ∿ 600.
$E'_x$ = Cis-P + Fractionated irradiation; $E'_o$ = Controls Cis-P
$E_x$ = Fractionated irradiation $E_o$ = Controls

related. Even if the rapidity in the development of an edema diverges within the two different treatment schedules, the mechanism for the observed phenomenon may be similar, namely damage to the cell membranes.

Membrane damage from ionizing radiation was earlier described both on an ultrastructural level (Flemming et al. 1968, Harris 1970, Willis 1966), and by measuring the leakage of proteins from blood vessels after irradiation (Song et al. 1966). Damage effects on the esophageal cell membranes could also be the reason for the observed loss of microridges with the formation of snakes and knobs. This process is easily observable and the number of S.A.K.s is easily calculated from SEM-pictures. Again the results with the different treatment schedules diverge where fractionated irradiation alone results in a positively significant dose-related number of S.A.K.s in relation to the dose within the irradiated area. The control area ($E_0$) remains fairly constant. The combined modality (cis-DDP + irradiation) indicates an increase in S.A.K.s both within the irradiated area ($E'_x$) and the control area ($E'_0$). However, the combined modality (cis-DDP + X-ray) does not increase the number of S.A.K.s within the irradiated area significantly, compared to fractionated radiation alone. It is assumed that damaged microridges, although reflecting the surfaces of the esophageal mucosa, give a good overview of the general condition of the mucosal epithelium. Therefore it seems as if the combined modality is well tolerated by this normal tissue. The administration of cis-DDP is usually combined with radiation therapy in a number of clinical studies. It is important to note that these studies have been designed without knowing the precise mechanisms for the interaction between the two modalities, the optimum timing or the dose-relationships. The timing between the administration of cis-DDP and irradiation may be crucial and it must be determined carefully in order to optimize the advantage of these interactions. Rapidly proliferating normal tissues like skin (Denekamp 1973, 1982), intestine (Withers and Elkind 1969), esophageal mucosa (Desmet and Tytgat 1974) and buccal mucosa (van der Schueren et al. 1983) are the normal tissues where the acute reactions can be the limiting factors in attempts to improve radiotherapy. These tissues seem to respond to radiation damage with a compensatory proliferation and acceleration of their proliferative capacity (Denekamp 1982). The recovery of these tissues seems to be mainly through repopulation, and the rapidity of this process may be dependent on the degree of damage (Fig. 16). Fig. 16 is supposed to reflect a hypothetical physiological situation in the turn-over rate of a mucous membrane. The figure at the top shows an area with a normal cell coating and a steady state of recovery. After an induced damage, either as the result of radiation or chemotherapy, there is an increase in cell loss which presumably stimulates the proliferation rate. A weak damage gives a slight loss of cells from the surface which is assumed to start a regeneration of basal cells (left part of the figure). A moderate aggravation of the damage would logically stimulate new formation of cells to a greater extent (middle part of the figure). However, the damage may be of such a grade that a transmission to the basal cells to be renewed has no response (right part of the figure) and the recovery rate gradually decreases. Although no such feedback mechanism has been proven electrophysiologically or anatomically it is widely accepted and in these experiments grade I damage corresponds best to the results obtained after fractionated irraditaion alone, grade II damage after treatment with cis-DDP + fractionated irradiation. This may explain the results obtained in this study. Although cis-DDP exerts a toxic effect on the esophageal mucosa with an edema, loss of microridges and an increased cell loss, the combination of cis-DDP and irradiation does not exert significantly more pronounced damage to the esophageal mucosa compared to fractionated irradiation alone. This is of direct clinical importance, since carcinoma of the esophagus is so far a disease with bad prognosis. The 5 year survival rate with radiation therapy is on average 5% (Earlam and Cunha-Melo 1980). Attempts to improve local control

and survival have recently been reported with a combination of radiotherapy and surgery (Hambraeus et al., 1987), and further with the addition of different kinds of chemotherapy sequential before radiation and surgery treatment (Mercke et al. 1984) or concomitant with radiation therapy (Keane et al. 1985). These different developments in treatment technique have the expectations of improving local control of the tumors and also the overall survival rate. However, the toxicity of treatment based upon normal tissue reactions must not exceed the therapeutic gain. Several experimental investigations must be pursued if the interactions between radiation and platinum complexes causing cell inactivation are to be optimized. It is necessary to examine the effects of the combined treatment on normal tissues.

## Conclusion

This research attempts to elucidate the mechanism of cis-DDP action on normal tissue and explores some possible avenues that can be followed to prevent its toxic side effects. Fractionated irradiation of the rabbit's esophagus results in a dose-dependent edema of the esophageal mucosa, loss of microridges on the surface and an increased cell loss. The addition of cis-DDP day 1 on the fractionated radiation schedule did not significantly increase the effects observed by fractionated radiation alone. A damage effect could be observed with an edema, damaged microridges and increased cell loss. However, with increasing time the esophageal mucosa recovered.

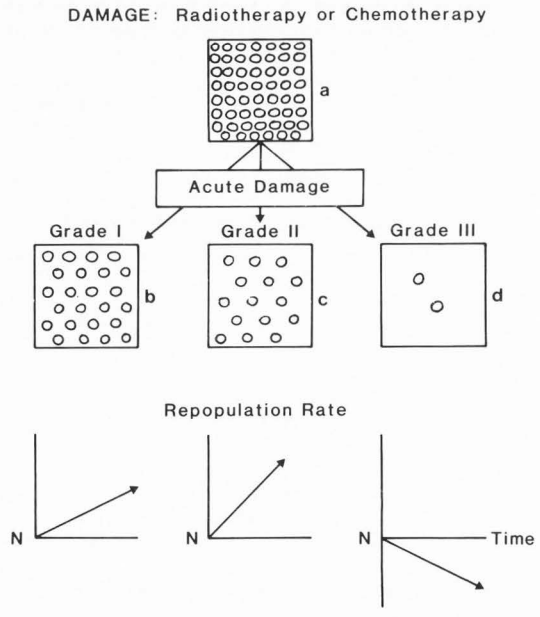



**Figure 16.** Damage and repopulation.
a = the number of cells in normal steady state;
b = the number of cells after damage grade I;
c = the number of cells after damage grade II;
d = the number of cells after damage grade III.

a = N (normal stimulation);
stimulation b > a;
stimulation c > b >> a;
stimulation d > c >> b >>> a.

## Acknowledgements

We wish to express our gratitude to Miss Marianne Palmegren, who did all the preparations for SEM- and TEM-examinations, at the oncology - EM laboratory. The institute of Zoology, University of Lund, Sweden kindly provided the facilities for scanning and transmission electron microscopy.

This work was supported by grants from John and Augusta Persson's Foundation for Scientific Medical Research, from the Medical Faculty of the University of Lund, from Lund's Hospital Research Foundations, and B. Kamprad's foundation.

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## Discussion with Reviewers

K. Carr: How was the dose of cis-Platinum chosen? Have different doses been tried? Have you tried fractionated doses of this treatment?
Authors: In this paper we have referred to clinical investigations where the dose of cis-DDP is in $mg/m^2$, administered every third week. This has been converted to a corresponding dose for rabbits. The current paper is part of a major research program, where our next step will be treatment with fractionated doses of cis-DDP.

K. Carr: Why was the quotient $E_x/E_0$ used to plot the results?
Authors: In control animals $E_x/E_0 = 1$. LM investigations were performed where the height of the mucosal epithelium was measured from 1-2 um thick sections and the quotient $E_x/E_0$ is believed to represent the edema of the mucosal epithelium in the irradiated area.

J. Reitan: In current treatment protocols with preoperative treatment radiation doses of, e.g., 36 Gy combined with cis-DDP seems to be fairly well tolerated. Do you think that the reactions after cis-DDP is maximal or could it be further increased if the radiation doses had been higher? Do you have any observations beyond ten days?
Authors: Different clinical studies have been designed without knowing the precise mechanism for the interactions between cis-DDP and radiation, the optimum timing or the dose relationships. The timing between administration of cis-DDP and irradiation may be crucial and the results differ from one tissue to another. This must be determined carefully in order to optimize the advantage of these interactions. Unfortunately we do not yet have observations beyond ten days.