

Tubuloreticular structures were found inside endoplasmic reticulum from endothelial cells of the tumor vessels (Figures 1 and 2). The branched tubules which measured 220–250 Å in cross section, seem at some points to be continuous with the cisternal membrane and are associated with intracisternal dense material (Figure 2). No similar structures were seen inside tumor cells.

- ⁴ K. BLINZINGER, J. SIMON, D. MARGRATH and L. BOULGER, *Science* **163**, 1336 (1969).
⁵ F. GYÖRKEY, J. G. SINKOVICS and P. GYÖRKEY, *Cancer* **27**, 1449 (1971).
⁶ Z. SCHAFF, D. W. BARRY and P. M. GRIMLEY, *Lab. Invest.* **29**, 577 (1973).
⁷ W. A. JENSEN, *J. Ultrastruct. Res.* **22**, 296 (1968).
⁸ J. M. BASSOT, *J. Cell Biol.* **37**, 135 (1966).
⁹ S. CHANDRA, G. E. MOORE and P. M. BRANDT, *Cancer Res.* **28**, 1982 (1968).
¹⁰ J. R. BARINGER and J. F. GRIFFITH, *Science* **163**, 1336 (1969).
¹¹ E. BUCCIARELLI, G. F. RABOTTI and A. J. DALTON, *J. natn. Cancer Inst.* **38**, 359 (1967).
¹² H. L. MOSES, P. R. GLADE, J. A. KASEL, A. S. ROSENTHAL, Y. HIRSHAUT and L. N. CHESIN, *Proc. natn. Acad. Sci., USA* **60**, 489 (1968).
¹³ S. CHANDRA, *Lab. Invest.* **18**, 422 (1968).
¹⁴ The author thanks to Prof. Dr. J. M. CORTEZ PIMENTEL and to Dr. L. LEITE NORONHA for help and advise.

These structures were thought to represent the nucleoprotein strands of paramyxovirus¹, polio virus⁴, and to be related with type C oncornavirus⁵. Recent ultrastructural cytochemical evidence does not support their direct viral significance^{2,6}, and together with their detection in normal and sometimes highly differentiated cells^{7–9}, it seems to indicate that they are a cell organelle. However their presence in viral infections^{4,10}, virus-induced tumors¹¹, and virus-infected cell lines^{12,13} raises the question of their aetiological significance to viral diseases and makes them a suggestive finding in human neoplasia¹⁴.

Résumé. Les structures tubuloréticulaires ont été observées dans le reticulum endoplasmique des cellules endothéliales d'un adénome carcinomateux bronchique. Vu leur présence fréquente dans les infections virales, elles peuvent avoir une signification étiologique dans ces maladies-là, ce qui laisse supposer sa présence dans les cas de cancer humain.

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Effect of Irradiation on the Plasminogen Activator Content in Rat Vessels

The fibrinolytic activity of vessels has been found to be confined to the endothelium of vasa vasorum in the adventitia¹. Studies in tissue culture have demonstrated that fibrinolytic activators also are synthesized in the vessel walls². Irradiation damages the vessel endothelium and leads to fibrotic changes of the vessel walls³ and increased frequency of thrombosis⁴. Irradiation has also been shown to decrease enzyme synthesis⁵.

To elucidate the effect of irradiation on the plasminogen activator content of the vessel walls, we compared the fibrinolytic activity histochemically in rat vessels before and after irradiation.

30 Sprague-Dawley rats of uniform age and weighing about 250 g were used. 20 of the animals were irradiated with 1000 rads in a single dose. 10 of them were killed and examined after 1 month and 10 after 2 months. The remaining 10, non-irradiated animals, served as controls. The heart, abdominal aorta and iliac veins were removed. The specimens were immediately frozen in expanding CO₂, packed hermetically in Parafilm® to prevent drying, and stored at –60 °C until examined.

Cryostat sections, 8 µm thick, were cut and collected on cleaned glass slides. Sections of the heart were placed in planes through the coronary sulcus. The fibrinolytic activity was determined histochemically with the method of TODD⁶, as modified and graded in arbitrary units by PANDOLFI et al.⁷. Four slides of every sample were incubated for 15, 30, 45 and 60 min, respectively. The

- ¹ M. PANDOLFI, Thesis, Berlingska Boktryckeriet, Lund (1969).
² B. ÅSTEDT and M. PANDOLFI, *Revue Eur. Etudes clin. Biol.* **17**, 261 (1972).
³ R. H. THOMLINSON, *Br. med. Bull.* **29**, 29 (1973).
⁴ P. RUBIN and G. CASARETT, *Clinical Radiation Pathology* (W. B. Saunders Company, Philadelphia 1968), vol. 1, p. 47.
⁵ D. J. MARCIANI and B. M. TOLBERT, *Biochim. biophys. Acta* **302**, 376 (1973).
⁶ A. S. TODD, *J. path. Bact.* **78**, 281 (1959).
⁷ M. PANDOLFI, I. M. NILSSON, B. ROBERTSON and S. ISACSON, *Lancet* **2**, 127 (1967).

Comparison between the fibrinolytic activity in vessel walls of non-irradiated and irradiated rats (Arbitrary units. Median value and range)

Group	Vena iliaca sin.	Vena iliaca dx.	Aorta	Coronary vessels
(A) Non-irradiated	2 (1.5–3)	2 (1–4.5)	3.25 (1–4.5)	2.25 (1.5–5)
(B) Irradiated (1000 rads) and killed after 1 month	1.5 (0–2.5) ^b	0.5 (0–3) ^a	1.5 (0.5–3.5) ^a	1.75 (0.5–2.5)
(C) Irradiated (1000 rads) and killed after 2 months	0.5 (0–1) ^c	0.5 (0–1.5) ^c	0.5 (0–1.5) ^c	1 (0.5–1.5) ^c

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$.

fibrinolytic activity of different groups of veins was compared by the Wilcoxon rank sum test⁸.

The results are given in the Table. In the rats, irradiated with 1000 rads and killed 1 month later (group B), the fibrinolytic activity in their vessel walls tended to be decreased. In the group C, killed 2 months after irradiation, the decrease was highly significant ($p < 0.001$).

The findings indicate a decrease in the activity of the cells producing the plasminogen activator enzyme. This is in agreement with findings by SVANBERG et al.⁹ of a decreased fibrinolytic activity in a case of ovarian tumour examined before and after irradiation. The decreased activity is probably a manifestation of a damage to the vascular endothelium. It is known that

this is followed later by fibrotic changes in the vessel walls, which may explain why the decrease in the fibrinolytic activity was larger after 2 months than after 1.

Tumours possess fibrinolytic activity which can be related to their vascularity⁹. The fibrinolytic activity in the vessel walls is of importance for the patency of vessels. The present experimental findings may help to explain the complex effect of irradiation on tumour growth.

Zusammenfassung. Bei bestrahlten Ratten wurde die fibrinolytische Aktivität verschiedener Gefäße histochemisch untersucht und mit denen einer Kontrollgruppe verglichen. Es wurde eine Herabsetzung der fibrinolytischen Aktivität der bestrahlten Gefäße gefunden, welche durch Beschädigung der Gefäßendothelzellen erklärt werden könnte.

⁸ W. J. DIXON and F. J. MASSEY, *Introduction to Statistical Analysis* (McGraw-Hill Inc., New York 1957), p. 290.

⁹ L. SVANBERG, F. LINELL, M. PANDOLFI and B. ÅSTEDT, *Acta path. microbiol. scand.*, in press (1974).

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Enhancement of Hemopoietic Colony-Formation in the Mouse Peritoneal Cavity by the Treatment with Carbon Particles

Accumulated data suggests that stromal elements play an important role in hemopoietic regeneration¹⁻⁶. In the previous study we have also been able to demonstrate that hemopoietic stem cells which had been irradiated *in vitro*, recovered more effectively in the RES-blockaded host mice than in the control hosts⁷ and have concluded that this phenomenon could be responsible for the increased survival and enhanced recovery of hemopoietic system of the irradiated mice by the blockade of RES with carbon particles⁸⁻¹¹. These findings strongly suggest that the RES-blockaded mice provide a favorable micro-environment for the slightly damaged hemopoietic stem cells to recover. Concerning the possible control mechanism through some humoral factor, METCALF et al.^{12,13} reported that the release of the colony-stimulating factor was greatly stimulated by the treatment of animals with bacterial endotoxin. It may be possible that carbon particles have a similar effect on the release of such factor(s), since both carbon particles and bacterial endotoxin are known to be most effective radioprotectants and stimulate hemopoietic recovery after irradiation^{14,15}.

In order to clarify whether the above-mentioned favorable effect of carbon-treatment of host mice on the recovery of irradiated colony-forming cells is due to humoral factor(s), extramedullary hemopoietic colony-formation was studied in the host mice either treated with carbon particles or with bacterial endotoxin.

Materials and methods. Details of the method for colony-formation on macrophage layer were reported by SEKI¹⁶. A cellulose acetate (CA) membrane (20 × 20 mm) was inserted into the peritoneal cavity of 9-week-old male mice of DDD strain. 4 days later, they were injected i.v. either with 10 mg of carbon particles (Pelikan India ink C11/1431 a, Germany) or with 20 µg bacterial endotoxin (lipopolysaccharide B from *E. coli* O 111:B4, Difco, USA). 24 h after the treatment, the animals were irradiated with 680 R of X-rays, and then injected i.p. with

freshly harvested bone marrow cells¹⁰. 7 days after the injection of the bone marrow cells, the CA membranes were taken out and colonies formed on the macrophage layer were counted after the visualization procedure by the peroxidase reaction. In order to investigate the effect of host microenvironment on the recovery of irradiated hemopoietic stem cells, other groups of host mice, into which the CA membrane was also inserted, were exposed to 480 R of X-rays, and then injected with the bone marrow cells. 1 h later they were irradiated again with 200 R of X-rays. Thus, the host mice received 680 R of X-rays, while the grafted bone marrow cells were exposed only to the second dose of 200 R *in situ*. Other procedures were the same as in those for colony-formation with the

¹ N. S. WOLF and J. J. TRENTIN, *J. Cell Physiol.* 75, 225 (1970).

² M. BERAN, *Strahlentherapie* 144, 719 (1972).

³ J. H. HENDRY and L. G. LAJTHA, *Radiat. Res.* 52, 309 (1972).

⁴ W. FRIED, W. CHAMBERLIN, W. H. KNOSPE, S. HUSSEINI and F. E. TROBAUGH JR., *Br. J. Haemat.* 24, 643 (1973).

⁵ A. MANIATIS, M. TAVASSOLI and W. H. CROSBY, *Blood* 38, 569 (1971).

⁶ R. S. McCUSKEY, H. A. MEINEKE and S. F. TOWNSEND, *Blood* 39, 697 (1972).

⁷ K. J. MORI and Y. ITO, submitted to *Radiat. Res.*

⁸ K. J. MORI and S. NAKAMURA, *Experientia* 26, 1386 (1970).

⁹ S. NAKAMURA, *Radiat. Res.* 52, 130 (1972).

¹⁰ K. J. MORI, *Radiat. Res.* 56, 494 (1973).

¹¹ S. NAKAMURA and K. J. MORI, *J. Radiat. Res.* 75, 14 (1974).

¹² S. H. CHAN and D. METCALF, *Blood* 40, 646 (1972).

¹³ D. METCALF, *Immunology* 21, 427 (1971).

¹⁴ W. W. SMITH, I. M. ALDERMAN and R. E. GILLESPIE, *Am. J. Physiol.* 191, 124 (1957).

¹⁵ W. W. SMITH, G. BRECHER, S. FRED and R. A. BUDD, *Radiat. Res.* 27, 710 (1966).

¹⁶ M. SEKI, *Transplantation* 16, 3 (1973).