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RADIATION EFFECTS ON THE FINE BLOOD VESSELS IN ABDOMINAL ORGANS OF MICE

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In radiation therapy of malignant diseases, the relative radiation effects on the tumour and on the normal tissues play an important role for the curability (PATERSON 1963). The tolerance limit of the normal tissues may be due to the sensitivity of the vascular system. Irradiation of tissues causes early morphologic and functional changes in the blood vessels. Also late radiation effects on the vascular system have been reported (KAWAMURA & FUJIWARA 1973, RUBIN et coll. 1964, ELLINGER 1957, JOLLES & HARRISON 1966, DEVIK 1955, HOLLAENDER 1956, MOSS & GOLD 1963, HASSLER & MOVIN 1966).

Various techniques for demonstration of the fine vascular structure as angiography, the India ink-gelatin mixture (KAWAMURA & FUJIWARA, ANGULO et coll. 1958, JEE & ARNOLD 1960, TIBOLDI et coll. 1968) and methods utilizing filling of the vessels with various contrast material (BISHTON & ROGERS 1950, HASSLER 1964) have been extensively used for recording of capillary injury and changes in the vascular structure after irradiation (KAWAMURA & FUJIWARA, RUBIN et coll., ELLINGER, JOLLES & HARRISON, DEVIK, HOLLAENDER, HASSLER & MOVIN, RUBIN & CASARETT 1966). In order to analyze the effect of radiation on the abdominal organs (small intestine, stomach, liver, kidney and spleen) the resin cast method (BATSON 1955, MURAKAMI 1971) was applied, and the results are now reported.

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Table 1

Number of animals examined after different periods. Animals that died before the planned observation time are given in parentheses

Dose	Days after irradiation	No. of mice	
		ddN	WH/HWT
5 Gy	1	3	3
	2	3	3
	3	3	3
	10	6	
	30	6 (2)	
10 Gy	1	6	3
	2	6	3
	3	6	3
30 Gy	1	6	
	2	6	
	3	6 (2)	
Non-irradiated controls	—	15	6

Material and Methods

Animals and irradiation technique. Seventy-two (including 15 controls) ddN albino strain mice (females, average weight 25 g) and 24 (including 6 controls) WH/HWT albino strain mice (males, weight about 20 g) were used (Table 1).

The animals were divided into four groups: non-irradiated controls and animals irradiated with 5, 10 and 30 Gy, respectively. The irradiated animals received one single treatment with a gamma ray beam from a telecobalt unit (SSD 80 cm, field size 30 cm × 30 cm, dose rate 0.45 Gy/min). The radiation beam was calibrated with an Ionex dosimeter. The animals were killed after different periods. Two animals died during the experimental period.

Preparation of the resin. The base of the resin was methyl-methacrylate monomer. Before injection, the resin had to be freshly prepared. First, 2,4-dichloro-benzoyl peroxide (catalyst) was added to the monomer up to a final concentration of 2 per cent. The mixture was then warmed to about 75°C and thereafter rapidly cooled. Then benzoyl peroxide (catalyst) was added, and just before the injection into the animal, dimethylanilin (accelerator) up to 2 per cent.

This preparation technique of resin was a modification of the method of MURAKAMI.

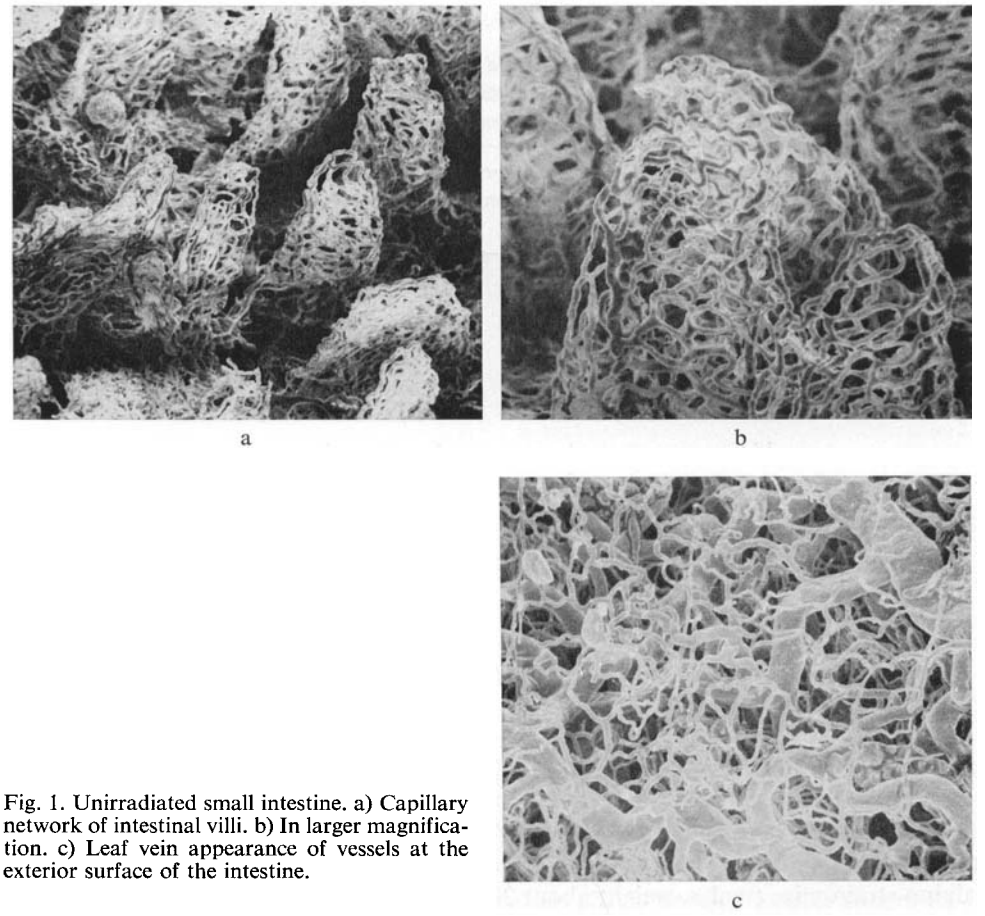

Fig. 1. Unirradiated small intestine. a) Capillary network of intestinal villi. b) In larger magnification. c) Leaf vein appearance of vessels at the exterior surface of the intestine.

Resin injection and corrosion casting. The animals were anesthetized with ethylether and decapitated, and then irrigated with Ringer solution through the aorta. The prepared mixture (subpolymerized state) was injected into the thoracic aorta under moderate pressure. The injected animals were placed in hot water (60–70°C) and kept at about 70°C in an incubator. After a few hours, sodium hydroxide (about 20 %) was added for corrosion of the soft tissues. The resin casts obtained were washed and dried with ethyl alcohol in air.

Scanning electron microscopy. The dried whole body resin cast was dissected and sliced. The sliced resin casts were fixed on an aluminium block with a silver paste, and then exposed to vacuum evaporation with carbon and gold. These casts, which were coated by electron dense materials, were examined and photographed with a scanning electron microscope (model MSM-4, Hitachi, Japan). By this method not only the arterial system but also the capillary and venous systems were demon-

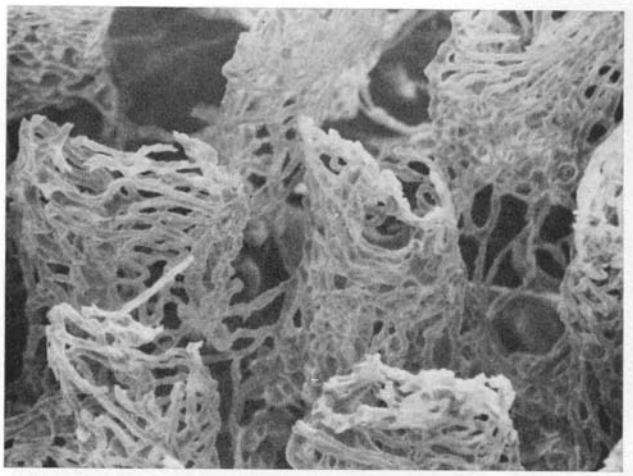

Fig. 2. The capillary network of the intestinal villi at 30 days after irradiation with 5 Gy. Capillaries at the top of villi partially destroyed.

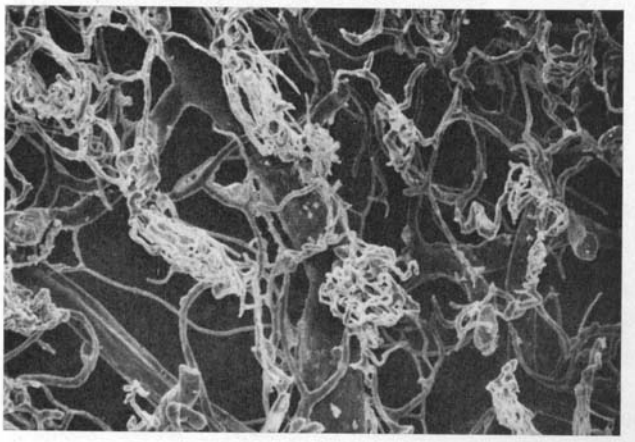

Fig. 3. Capillary network of the intestinal villi at 3 days after irradiation with 10 Gy markedly reduced.

strated. It is difficult to discriminate these two systems at their terminal regions, but as a rule the venous system is possible to recognize since the veins are thicker in the postcapillary region.

Results

No significant differences were observed between the two strains of mice.

The resin cast method could distinctly demonstrate the shape of the small blood vessels contrary to other methods. The characteristic appearance of the vascular network is illustrated in the figures.

Small intestine. The vascularity of the small intestine can be classified into 4 groups: the mucosal, submucosal, muscular and subserosal vessels. By the scanning electron microscope three of these groups could be observed, namely the mucosal, the muscular and the subserosal vessels. The mucosal vessels formed a characteristic

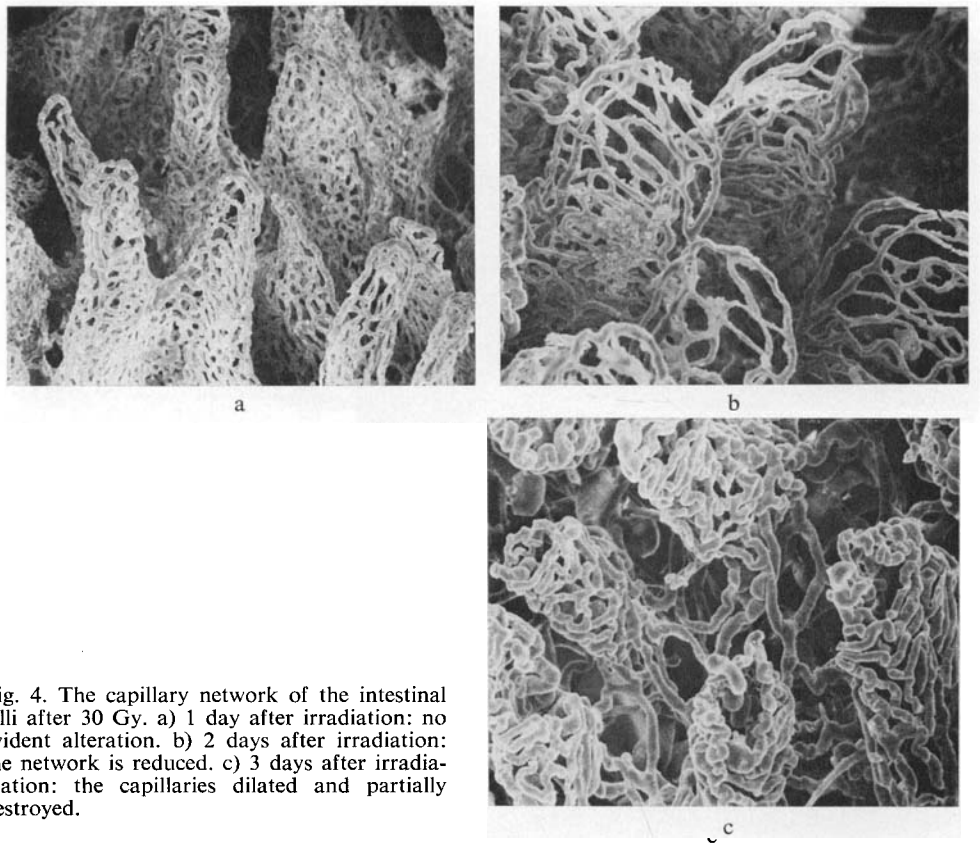

Fig. 4. The capillary network of the intestinal villi after 30 Gy. a) 1 day after irradiation: no evident alteration. b) 2 days after irradiation: the network is reduced. c) 3 days after irradiadiation: the capillaries dilated and partially destroyed.

network. Its general appearance was the same as that of the intestinal villi, with a regular reticular arrangement (Fig. 1). The muscular and subserosal vessels branched off from the large blood vessels at the exterior surface of the intestine in a way similar to leaf veins.

In contrast to the non-irradiated mice, both the general arrangement of the vessels and the shape of the capillaries themselves were changed in the irradiated animals. At 10 days and 30 days after a single whole body irradiation with 5 Gy, the capillary network at the top of the intestinal villi had been partially destroyed (Fig. 2). At 3 days after 10 Gy (Fig. 3) and 2 days after 30 Gy (Fig. 4 b) similar alterations were observed and the capillaries were reduced in number. These changes were more extensive than after 5 Gy, but the diameter of the capillaries was rather uniform after the different exposures. At 3 days after 30 Gy, marked vascular dilatation had occurred, and the capillary diameters were not uniform (Fig. 4 c). The vascular network at the top of the intestinal villi had been partially destroyed. The vessels at the exterior of the intestine were not changed. No obvious alterations could be found at 1, 2 and 3 days after 5 Gy and at 1 and 2 days after 10 Gy and at 1 day after 30 Gy.

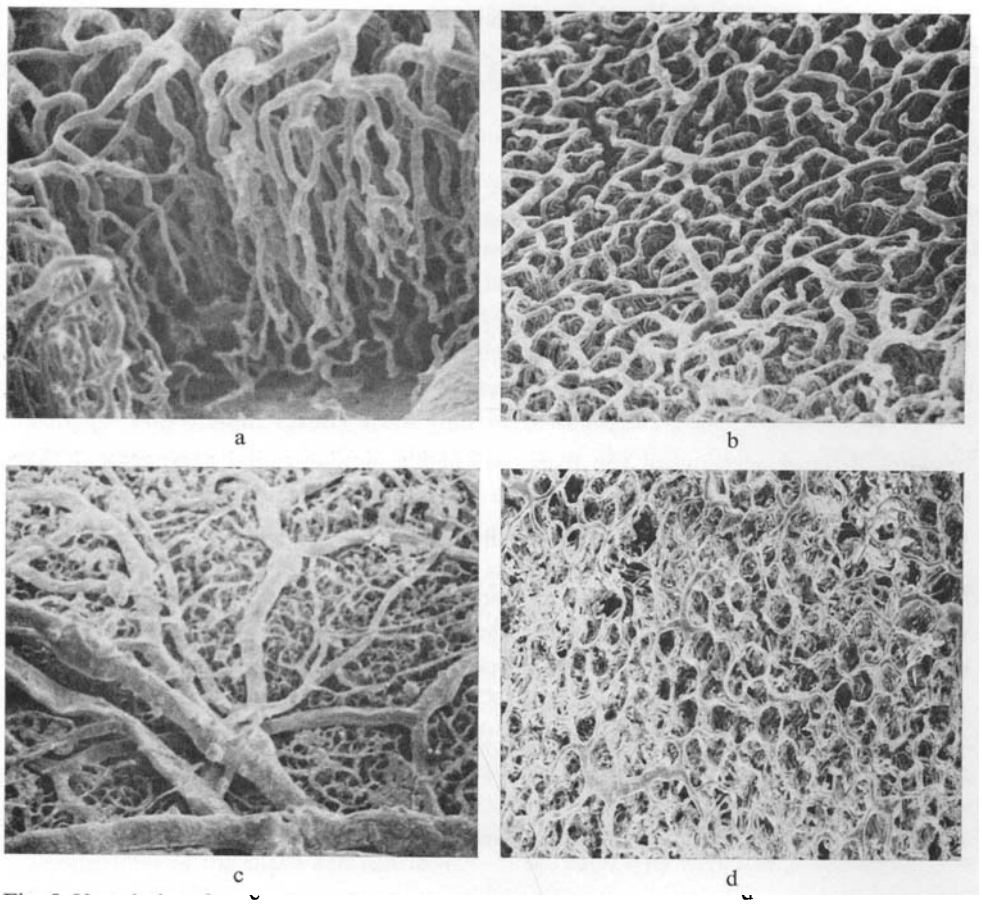

Fig. 5. Vascularity of stomach. a) Capillaries arranged perpendicularly to the stomach wall. Non-irradiated control. b) Honeycomb appearance of vessels at inside of stomach. c) Leaf vein appearance of vessels at outside of stomach. Non-irradiated control. d) Honeycomb appearance of vessels at 3 days after irradiation with 10 Gy. The vessels are narrower than normal.

Stomach. The vascular system of the stomach can be divided into 5 groups: the mucosal vessels, the vessels around the muscularis mucosae, the submucosal, muscular and subserosal vessels. The mucosal, muscular and subserosal vessels were possible to observe. The mucosal vessels were arranged perpendicularly to the stomach wall, and the top of these vessels form a characteristic network with a honeycomb-like shape. The muscular and the subserosal vessels had an appearance similar to the branched vessels of the small intestine. At 3 days and 2 days after 30 Gy and 3 days after 10 Gy, the perpendicularly arranged vessels seemed to be coarser and the honeycomb-like vessels thinner than normally, but these changes were not quite certain (Fig. 5).

Liver. The vessels at the liver surface were, in the non-irradiated mouse, arranged radially from the central veins, and had a characteristic shape. Also the vascular

Table 2

Irradiation effects on the capillaries of different organs. Uncertain effects are given in parentheses

	Days after irradiation at										
	5 Gy					10 Gy			30 Gy		
	1	2	3	10	30	1	2	3	1	2	3
Intestine	–	–	–	+	+	–	–	+ +	–	+	+ +
Stomach	–	–	–	–	–	–	–	(+)	–	(+)	(+)
Liver	–	–	–	–	–	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	–	–	–	–	–
Spleen	–	–	–	(+)	(+)	–	–	–	–	–	–

network in cross sections of the liver cast had a similar radial arrangement. The vascular distribution in the liver was more dense than in the other organs. No radiation effects could be distinctly observed in any of the irradiated groups.

Kidney. At the surface of the kidney, the vessels were arranged as a reticular network. At the interior part of the kidney, the vessels ran radially from the renal pelvis through the medulla to the cortex. In perpendicular sections these bundles of blood vessels had a honeycomb-like structure. Through the 'spaces of the honeycomb' other bundles of blood vessels passed. In the cortex, branching vessels were demonstrated and also capillary structures constituting the renal glomeruli. The vascularity of the kidney was very dense. No obvious radiation effect on the renal vessels was found in any of the experimental groups when compared with non-irradiated controls.

Spleen. The vascular system of the spleen consisted of branching vessels and irregular lumps with sinusoid-like vessels. The vascularity at 10 days and 30 days after 5 Gy seemed to be somewhat reduced. These observations were, however, not quite evident, and it was not possible to exclude artefacts introduced by the high pressure at which the resin must be infused into the spleen.

The capillary changes due to irradiation are summarised in Table 2.

Discussion

The technique applied, the resin cast method, demonstrated the fine vascular morphology better than other, previously used methods (ANGULO et coll., JEE & ARNOLD 1960, TIBOLDI et coll., BISHTON & ROGERS, HASSLER, RUBIN & CASARETT 1966). Irradiation of the different tissues and organs resulted in changes of their structure which modified the appearance of the vascularity. The changes of the vessels might be considered as secondary to injury of the surrounding structures. A direct radiation effect on the capillaries may also occur, but is not possible to prove

with the methods used. An indication that such an effect may exist is known. A capillary cast of the intestine made as soon as 3 days after irradiation with 30 Gy demonstrated irregular thickness and widening of the capillary diameters compared to the control group.

Previously, early physiologic radiation effects on the vessels have been observed as permeability changes demonstrated by use of dye or colloid but not as morphologic changes. Several authors have reported that the early changes of the capillaries are of a purely functional type, but that apparent morphologic changes can be observed only at a later stage and after large doses of radiation (ELLINGER, HOLLAENDER, HASSLER & MOVIN, RUBIN & CASARETT 1968).

Necrosis of important tissues after irradiation causes problems in the clinical situation. Injury to the capillaries has been registered as one of the main causes of necrosis. The present results show that early changes of the vascularity of several tissues may occur, but further investigations of the late radiation effects on the vascularity are motivated.

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SUMMARY

The capillary networks of normal and irradiated abdominal organs of mouse were investigated by a resin cast technique. The structure of the capillary system had characteristic appearances. Radiation effects on the fine vascular structures were demonstrated from one to 30 days after a single dose of 5 to 30 Gy whole body irradiation. Prominent morphologic abnormalities of the shape and distribution of the capillaries were identified, especially in the small intestine.

ZUSAMMENFASSUNG

Das Kapillarsystem der Abdominalorgane von normalen und bestrahlten Mäusen wurde mit Hilfe einer Harzgusstechnik untersucht. Die Struktur des Kapillarsystems hatte ein charakteristisches Aussehen. Die Strahlungseffekte auf die feinen Gefässe wurden zwischen einem und 30 Tagen nach einer einmaligen Dosis von 5 bis 30 Gy Ganzkörperbestrahlung beobachtet. Deutliche morphologische Veränderungen des Aussehens und der Verteilung der Kapillaren, besonders im Dünndarm, wurden festgestellt.

RÉSUMÉ

Le réseau capillaire d'orgages abdominaux de souris normales et de souris irradiées a été étudié par une technique de moulages par une résine. La structure du système capillaire a des aspects caractéristiques. L'effet des radiations sur les fines structures vasculaires a été mis en évidence de 1 à 30 jours après une dose unique de 5 à 30 Gy d'irradiation corporelle totale. Des anomalies morphologiques importantes de la forme et de la distribution des capillaires ont été identifiées, en particulier sur l'intenstin grèle.

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