Indirect Induction of Vascular Damage by X-Irradiation

By Charles K. Levy, Ph.D.

The increased vascular permeability and hemorrhagic defects which have been observed following total body irradiation have been attributed to many causes. Cronkite et al.1,2 correlated these changes with the well demonstrated thrombocytopenia3 which occurs a few days after exposure to whole-body ionizing radiation. However, there are a considerable number of reports which suggest that the etiology of these vascular changes might be attributed to one or more of the following: (1) direct damage to the vascular endothelium and perivascular connective tissue;4,5 (2) hypoprothrombinemia;6 (3) a decrease in the concentration of circulating fibrinogen and damage to the reticuloendothelial system;7 (4) an increase in circulating histamine;8,9 (5) the destruction of mast cells and hyperheparinemia;10 (6) the release of some yet uncharacterized anticoagulant;10,11 and (7) vasodilation,12 which would increase the tension on the walls of the vessels, thus increasing permeability and vascular fragility. All may be implicated as contributory factors acting either independently or synergistically in the postirradiation vascular syndrome. The present studies were undertaken to clarify, if possible, the role of humoral factors in the pathogenesis of vascular disturbances following total body irradiation.

Methods

Golden hamsters (Mesocricetus auratus) were anesthetized with an intraperitoneal injection of sodium pentobarbital (10 mg./100 Gm. body weight). Both cheek pouches were everted and pinned out. One pouch was then shielded with a lead cover (8 mm. thick) and an area (500 mm.2) of the abdomen was also shielded with the same material. Each animal was given 1,000 roentgens whole body x-irradiation of the following characteristics: 145 Kk, 6.5 ma., 0.5 mm. aluminum filter inherent in the tube, 30 cm., 35 y/minute.

The moccasin (Agkistrodon piscivorus) venom test of Fulton et al.13 was used to measure vascular fragility by means of induced petechial formation. In this test, anesthetized hamsters had both cheek pouches everted and pinned out to allow simultaneous transillumination and direct microscopic observation of the blood vessels of the pouch. Each pouch was then injected with 0.02 ml. of fresh 0.01 per cent moccasin venom dissolved in physiologic saline; petechial counts were made at 34-hour intervals up to 1½ hours, at which time the animals were returned to their cages. All tests were run on the fourth day after irradiation.

These same animals were used again on the eighth postirradiation day to measure changes in dermal permeability by means of the technic of Upton and Gude.14 Changes in dermal permeability are not necessarily related to changes in vascular fragility, but the studies of Upton and Gude suggest the possibility that the two are related. Eight days after irradiation, each animal was anesthetized with sodium pentobarbital by intraperitoneal injection at a site far removed from the area where 0.05 ml. of 0.5 per cent Evans blue was injected intradermally into both sides (irradiated and shielded) of the shaved abdomen. Several booster doses of sodium pentobarbital were needed to keep the animals immobile during the course of the experiment, since body movements could alter the rate of diffusion of the dye. The elliptical-shaped blue dye wheals were measured by means of a vernier caliper. The longest diameter (D) and shortest diameter (d) were measured in millimeters at intervals of 1, 2 and 3 hours and the areas of coloration were then calculated by the formula:

\[ \frac{(D) \times (d)}{2} = \text{Area}^{15} \]

Results

By means of the petechial susceptibility test, both the shielded and unshielded pouches of the irradiated animals showed a marked increase in the number of petechiae when compared to the counts made on the right and the left pouches of the unirradiated controls. However, there was no statistically significant difference between the shielded and unshielded pouches of the irradiated hamsters (fig. 1).
Figure 1

Number of petechiae produced in the everted hamster cheek pouch after injection with moccasin venom is compared for the control group C, the shielded pouch of the irradiated animal S and the unshielded pouch of the irradiated animal I. Each dot represents 1 pouch. A count of 150/pouch constituted the end point because difficulty in counting was encountered beyond that point. Both S and I show increase over the control C, but do not differ significantly from one another.

As an additional control, several animals had only 1 everted pouch irradiated and the remainder of the animal shielded. The results of these tests showed that the irradiated pouch had a greater hemorrhagic susceptibility than the unirradiated pouch. This observation agrees with the response reported by Fulton et al.8–13 Thus it appears that total body irradiation (except for small shielded areas) produced hemorrhagic susceptibility, even in the shielded sites.

The dye wheal surface areas of the shielded and unshielded sides of the irradiated animals both exceeded the wheal surface areas of similarly injected controls, although no statistically significant differences were found between the values obtained from the shielded and the unshielded sides of the irradiated animals (fig. 2). The fact that on the eighth post-irradiation day there was an altered intra-

dermal permeability to Evans blue in the protected area comparable to the enhanced permeability found in the exposed area suggests that some diffusible or blood-borne humoral substance which alters permeability is released following massive irradiation.

Discussion

Alterations in permeability and vascular damage due to direct exposure to ionizing radiation have been reported by Brinkman et al.,16 Painter et al.,17 Rigdon and Curl,18 and Fulton et al.12 The evidence that the cells of the target organs are directly damaged by ionizing radiation is thus well demonstrated. In addition to the direct damage this study has demonstrated that some indirect factors are possibly involved in the hemorrhagic and permeability changes which follow irradiation. In general, these findings are in agreement with the reports of Jolles19 and Neumayr and Thurnher.20,21 Jolles19 reported that 2 fields exposed to irradiation which are close to one another exhibited greater inflammatory responses than were seen in 2 equally sized

Figure 2

Areas of the wheal produced by the intradermal injection of Evans blue (0.5 ml.) is shown for both male and female hamsters. The irradiated portion of the irradiated animal I and the shielded portion of the irradiated animal S both show enhanced permeability when compared with unirradiated control C. There was no statistically significant difference between S and I. Each spot represents the area 1 dye wheal.
fields further apart from one another. The clinical observations of Neumayr and Thurnher\textsuperscript{20,21} reported enhanced permeability in areas adjacent to the area exposed to x-irradiations. Such an indirect action has been termed a reciprocal vicinity effect. The mechanism by which this effect produces changes in permeability and vascular fragility remains obscure. There is some indication that humoral substances might be involved.\textsuperscript{8,11} Impairment of the cellular replacement homeostatic mechanism and damage to the clotting system\textsuperscript{1,3,6,7} have also been suggested. It would appear that both direct and indirect factors are involved in the postirradiation vascular trauma.

**Summary**

The changes in petechial susceptibility of the vessels of hamster cheek to the moccasin venom test have been compared in shielded and irradiated pouches of the same animal which had received median lethal doses of x-irradiation. There was no statistically significant difference demonstrable between the irradiated and controlled cheek pouches, but both showed a greater hemorrhagic response than was found in the cheek pouches of unirradiated control animals.

Similar results were found when the diffusion of intradermally injected Evans blue was used as an indication of x-irradiation damage. The shielded and the exposed areas both showed enhanced permeability to the dye when compared with the unirradiated control animals.

These results suggest that some indirect factor or factors are involved in the vascular and permeability changes following exposure to ionizing radiation.

**Acknowledgment**

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**Summary in Interlingua**

Le alterationes del susceptibilitate petechial del vasos in le gena del hamster al test a veneno de *Agkistrodon piscivorus* esseva studiate in animales pravemente subieti a doses medianmente latal de irradiation X in comparation con le grado de ille susceptibilitate in animales de controlo. Le alterationes in le gena del irradiate animales esseva studiate comparativemente con e sin protection del gena mensae durante le irradiation. Nulle statisticamente significative differencia del alteration del susceptibilitate esseva constatate in le irradiate animales inter le gena protegite e le gena non-protegite, sed ambas exhibiva un plus marcate responsa hemorrhagica quo le genas de non-irradiate animales de controlo.

Simile resultatos esseva obtenite quando le diffusion de injectiones intradermal de blau de Evans esseva usate como indication del damno irradiational. Tanto le protagite como etiam le non-protegite areas monstrava augmentos de permeabilitate pro le colorante in comparation con non-irradiate animales de controlo.

Iste resultatos suggere que un o plure factores indirecte es active in le effectuation del alterationes vascular e le alterationes de permeabilitate quo occurrre post exposition a radiation ionisante.

**References**


Errata for Volume VIII, Number I

Page 12: Footnotes for Table 4 should read as follows:
* = three normal volunteer subjects ages 26-36 (ref. 5) and 5 "essentially" normal subjects ages 26-69 (ref. 6).
† = nineteen normal and convalescent subjects without cardiopulmonary disease, ages 17-28 (unpublished).

Page 31: Table 1, first column: the word "isoelectric" should be in the lowest division. The word "depression" refers only to the second division. Second column: the word "baseline," at the top, refers to the columns of +, −, 0 signs, rather than to the figures.

Page 151: Legends for photographs B and D should be interchanged.

Page 153: Legends for figure 2: A refers to right lower; B middle lower; C lower left; D upper right; E upper center; and F upper left.

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