

Extraembryonic Vascular Deterioration in Irradiated Chick Embryo.* (23750)

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Extensive vascular damage in 6-day chick embryos has been observed following X-irradiation(1). Death within a week following median lethal doses of radiation received by 8 to 12-day-old embryos was characterized by hemorrhage and petechiae in the yolk sac and viscera(2). Extravasation of blood into the extraembryonic coelom of 4-day embryos was observed beginning $3\frac{1}{2}$ hours after exposure to 1200-20,000 r(3). The relative sensitivity of the chick embryo to radiation induced hemorrhage has recently been studied in terms of total dose, dose rate and stage of embryonic development(4). Hemorrhage was slight in 6-day embryos within a few hours following 600 and 800 r of 250 KVP X-irradiation. Twelve-day embryos, however, showed moderate hemorrhage following 600 r and extensive hemorrhage following 800 r (LD 95). The present work was undertaken as an attempt to quantitate the effects of X-irradiation upon the extraembryonic circulation of chick embryos.

Methods and materials. Twelve-day-old embryonated eggs were chosen because of the convenient size of the embryo, the extent of development of the extraembryonic circulation, and the marked hemorrhagic state which has been observed within a few hours after irradiation. A simple windowing procedure was devised which permitted rapid preparation of the embryonated eggs. A medially oriented 22 mm circular segment of shell was gently removed from the egg after being scored by a high speed motor-driven thin abrasive disc. The exposed shell membrane was then carefully removed without disturbing the underlying membranes. The shell aperture was then sealed with a circular silicone-grease rimmed microscope cover slip.

After the fenestrating process, the eggs were returned to the incubator for 24 hours. They were inspected under low power magnification just before irradiation, and any which appeared damaged were rejected. The remainder were coded and randomly divided into irradiation and control groups. The irradiation was delivered to the eggs supported on wire mesh in a circulating air incubator through a cover of one-sixteenth inch Plexiglass. The eggs were maintained at $38 \pm 1^\circ$ C throughout the experiment. The 800 r were delivered at 17 r/min. from a 250 kvp Picker Industrial generator utilizing the following factors: 250 kvp, 15 ma, Al. parabolic + $\frac{1}{2}$ mm Cu filter; HVL 2.25 mm Cu; 71 cm target to center of eggs.

Microscopic observations of the extent of injury to the allantoic and vitelline vessels were graded on an arbitrary scale of 5 ranks. Zero indicated no damage or visible impairment of function, while increasing injury was recorded by unit increments to 4, which represented maximum injury, loss of function or death. The condition of the major and minor blood vessels, the capillaries and the status of the blood flow in these 3 classes of vessels were scored. The eggs were graded under 20 magnifications with the window momentarily removed. Each egg was examined by 2 independent observers at $2\frac{1}{2}$, 4, 7, and 21 hours following irradiation. To preserve objectivity during the scoring period, the observers were not told whether the eggs were from the control or irradiated groups. The scores by experienced observers usually agreed within a half unit for any particular observation. Representative specimens were photomicrographed at 25 magnifications by reflected light during the course of the experiment.

Results. Vascular injury was detectable within a few minutes following irradiation. The course of injury as it develops between $2\frac{1}{2}$ and 21 hours following irradiation is shown in Fig. 1. The apparent lessening of

* Work performed under contract between Atomic Energy Commission and University of Rochester, administered by Dept. of Radiation Biology, School of Medicine and Dentistry.

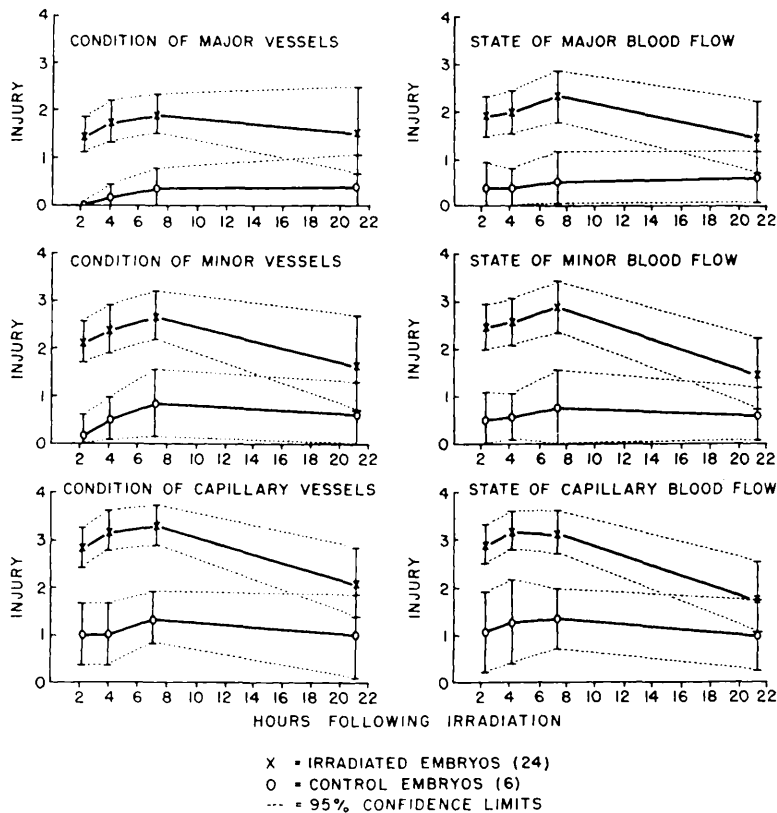


FIG. 1. Radiation injury to extraembryonic blood vessels of 12-day chick embryos.

damage after 7 hours is due to the procedure of recording only observations from surviving embryos. Non-survivors were recorded as 4 just prior to death after which the embryo was removed from the experiment (thus lowering the average value of subsequent observations on survivors, and decreasing the number of embryos in the group). By 21 hours 62% of the irradiated embryos were dead. None survived beyond 48 hours.

Injury was most pronounced in the capillaries. Extraembryonic vascular injury was observed simultaneously with the appearance of hemorrhage into the embryo. Blood flow through the vessels slackened and became sluggish in a sequence progressing from small to larger-sized vessels. By 3 hours even the largest vessels were affected. The general syndrome, as observed, was initiated first by a decrease in the blood flow, followed immediately by a constriction of vessel lumen. The vessels appeared to lose about half of their internal volume as the blood flow ceased.

Within an hour or two such vessels ruptured at several points of maximum constriction suggesting possible endothelial autolysis. Extravasated blood was observed in these regions. These observations suggested a weakening or defect of the vessel rather than a rupture resultant from normal pressure within the vessel. When many of the major vessels were affected, the embryo died. The time course varied from embryo to embryo but followed the above pattern until death. Each series of observations was completed within 30 minutes and recorded at the mean of the observation period. The prominent feature of these observations was the constriction and rupture of the extraembryonic vessels prior to the appearance of extravasated blood in that region. A possible contributing factor to the injury described for the extraembryonic vessels could have been the hemorrhage which has been noted within the embryo. These factors are subject to further investigation.

The condition of the blood vessels in non-

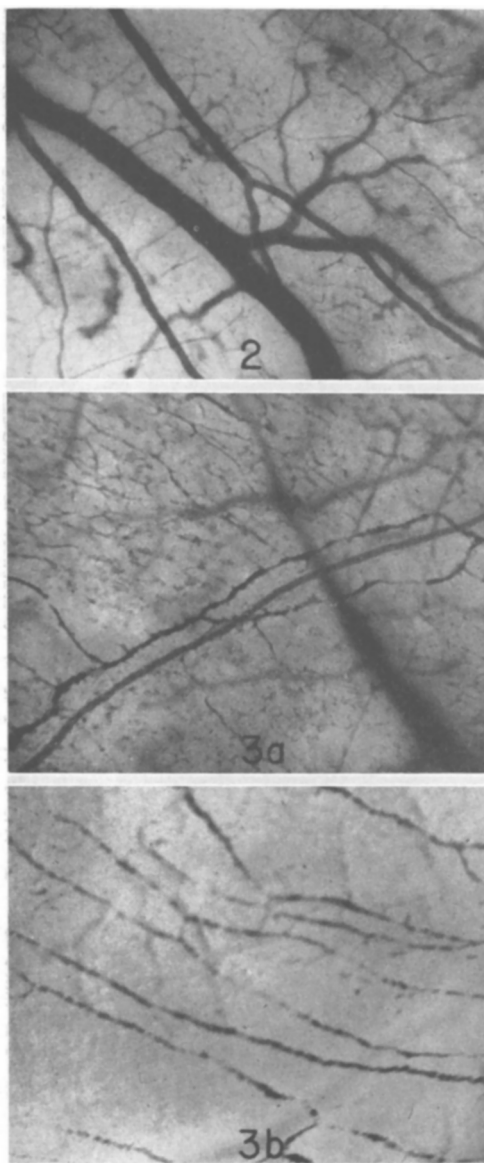


FIG. 2 and 3. Condition of extraembryonic blood vessels following 800 r. Fig. 2 = control. Fig. 3a = moderate deterioration 2.5 hr after irradiation. Fig. 3b = advanced deterioration 4 hr after irradiation. (15 magnifications.)

irradiated eggs is illustrated in Fig. 2. Capillary blood flow remained smooth and rapid throughout the experiment. Fig. 3a illustrates constriction and rupture of minor blood vessels at a time when many of the major vessels were still functional (2½ hours). Complete dissolution of the capillary network and rupture of many of the larger vessels prior to death of the embryo are illustrated in Fig. 3b (4 hours).†

Summary. Fenestrated eggs of 12-day chick embryos were given 800 r of 250 kvp X-rays. Injury to blood vessels of the extraembryonic membranes was detectable within minutes following irradiation. Deterioration was most rapid and extensive in the capillaries and least marked in the major blood vessels. Vascular disruption preceded the appearance of extravasated blood in that region. Intra-embryonic hemorrhage was also noted. Following irradiation the mortality was 62% at 21 hours and 100% by 48 hours.

† These observations have been repeated several times as teaching and student experiments with substantially the same results.

1. Strangeways, T. S. P., Fell, H. B., *Proc. Roy. Soc., London*, s. B., 1928, v102, 9.
2. Karnofsky, D. A., Patterson, P. A., Ridgway, L. P., *Am. J. Radiol. Rad. Ther.*, 1950, v64, 280.
3. Boland, J., *Br. J. Radiol.*, 1954, v27, 680.
4. Glasser, S. R., Brayer, F. T., *Fed. Proc.*, 1955, v14, 58.

Received October 30, 1957. P.S.E.B.M., 1958, v97.