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Received January 28, 1963. P.S.E.B.M., 1963, v112.

Radiation Technique for Production of Localized Myocardial Necrosis in the Intact Dog.* (28205)

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At present coronary ligation or cornary embolization is the principal technique for experimental production of myocardial infarction in laboratory animals(1,2). The former necessitates a thoracotomy, and the latter technique requires retrograde aortic catheterization. In an attempt to develop a more versatile method for production of localized myocardial lesions, the cardiac effects of a collimated beam of x-irradiation directed to the heart have been studied. The myocardium is considered a relatively radioresistant tissue, and the literature on cardiac irradiation is sparse(3,4,5,6). Warren summarized the cardiac effects of irradiation and described the non-specific changes of aseptic necrosis, hyaline fibrosis and obliterative vascular changes(4). In the aforementioned studies, the radiation dosage to the heart was less than 5,000 roentgens. Using a considerably higher dosage, a radiation technique for experimental production of localized myocardial necrosis is presented.

Methods. The study was performed on 12 adult dogs (15-20 kg). A 1,000 KVP General Electric Industrial X-ray Generator with a collimator attachment was used to deliver a roentgen beam 2.54 cm in diameter through the anterior chest wall to the heart of the dog. The radiation was delivered at a calculated rate of 210 roentgens per minute to the heart, and the half value layer of the beam was 3 mm lead. The calculated cardiac air dose of radiation for a given beam was between 2,500 to 20,000 roentgens depending on duration of irradiation. The portal of entry of the beam could be varied by simple positioning. During irradiation, the animals were anesthetized with intravenous sodium pentobarbital, 30 mg per kg of body weight.

Conventional 6 lead frontal plane electrocardiograms (ECG) were obtained before irradiation and almost daily during the first week following irradiation; thereafter, ECG tracings were taken at approximately weekly intervals until autopsy examination. The animals were sacrificed using a large intravenous dose of sodium pentobarbital and examined at arbitrary intervals after irradiation. Tissues were fixed in 10 per cent formalin, dehydrated and paraffin imbedded. Sections were stained with hematoxylin and eosin, and in a few instances phosphotungstic acid hematoxylin and phloxine-methylene blue stains were used.

Results. A preliminary study on 4 animals with varying doses of cardiac radiation (2,500, 5,000, 10,000 and 20,000 roentgens) demonstrated well-circumscribed myocardial necrosis by the 11th post-irradiation day in the animal receiving 20,000 roentgens. This latter dose was studied in greater detail. In each of 8 dogs, 20,000 roentgens were delivered through a single portal to the region of the apical cardiac impulse with the animal in the left anterior oblique position (30 degrees). The results are summarized in Table I.

* Supported by grants-in-aid from N. Y. Heart Assembly, by grant from U. S. Public Health Service, and contract with U. S. Atomic Energy Commission.

TABLE I. Canine Cardiac Irradiation—20,000 Roentgens.

	Autopsy day after irradiation				
	4	7	10	15	21
No. of cases	1	2	2	1	2
ECG	no change	no change	intermittent LBBB	Q, ST↑, T↓ in II, avF, III	Q, ST↑, T↓ in II, avF, III
Gross findings	normal	hemorrhagic induration of myocardium*	hemorrhagic softening and induration of myocardium	localized myocardial discoloration with endocardial thrombus formation	localized area of friable discolored myocardium; 100 cc pericardial effusion
Microscopic findings	"	coagulation necrosis	interstitial hemorrhage and muscle fiber necrosis	myocardial necrosis with early fibroblastic repair	myocardial necrosis with organization and fibroblastic repair

* Present in only one of the 2 dogs examined at this time interval.

The 3 dogs followed for more than 10 days after cardiac irradiation showed ECG evidence of acute myocardial infarction on tracings taken just prior to autopsy. Q waves, ST segment elevation and T wave inversion in leads II, avF and III developed between the 10th and 15th post-irradiation day.

Gross and microscopic evidence of myocardial necrosis was first evident in one of the 2 dogs examined on the 7th post-irradiation day, and thereafter present in all 5 dogs autopsied at later time intervals (10, 15 and 21 days). All lesions were about 3.5 × 3.5 cm in area and localized to the anterior and antero-lateral aspect of the left ventricle. The myocardial necrosis extended from epicardium to endocardium, and it was most extensive in the 21-day animals (Fig. 1). Microscopically, the myocardial lesion showed progressive evolutionary change from early coagulation necrosis at 7 days to necrosis and fibroblastic repair at 21 days. Near the margins of the lesions myocardial fibers were preserved around small blood vessels. Within the involved areas the media of small arteries was markedly thickened, and some of these vessels were completely occluded by poorly organized hyaline thrombi.

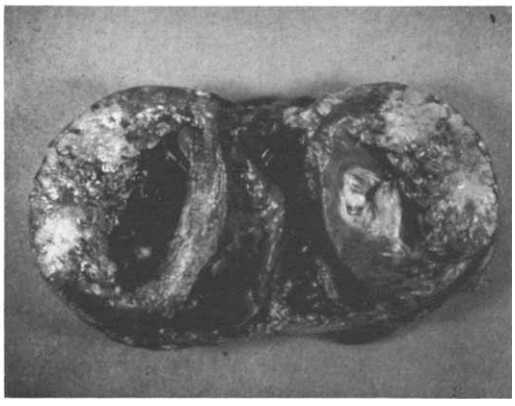

FIG. 1. Cross section of heart 21 days after irradiation showing well demarcated myocardial necrosis involving lateral aspect of left ventricle. Endocardial thrombus formation is present.

The x-ray beam produced a 3 × 3 cm skin lesion which varied from induration to necrotic ulceration depending on the post-irradiation time interval. The lingular portion of the left lung showed a hemorrhagic circular lesion (3 × 3 cm) in the position of the x-ray beam path. The remainder of the lung did not show radiation effect. Notable also was the absence of detectable damage in the posterior wall of the left ventricle through which the radiation beam must have passed. No alteration was observed in the esophagus or in the abdominal viscera, except for mild congestion of liver sinusoids in one 21-day dog.

Discussion. Selective cardiac irradiation has been shown to produce acute myocardial necrosis in the dog. The unique features of this technique include (1) ease of production of the lesion in a closed-chest animal; (2) the well-circumscribed nature of the necrosis with its location isolated to the anterior portion of the free wall of the left ventricle; and (3) the gradually progressive nature of the evolving necrosis beginning about 7 days after irradiation.

To our knowledge, cardiac radiation effects in the 20,000 roentgen dose range have not been previously studied. In general, histopathologic changes seen in irradiated tissues include cellular and intercellular breakdown, as well as alterations in endothelial linings of small blood vessels(3,7). It appears that vascular damage is of fundamental importance in production of aseptic necrosis(7). In the present study, microscopic findings in the heart suggest a primary vascular lesion with secondary ischemia and infarction. The histopathologic similarity of the cardiac lesion produced by irradiation to human myocardial infarction is striking and deserves emphasis.

The application of the present technique to the experimental study of myocardial infarction is self-evident. With this technique the size and location of the myocardial necrosis could be varied by simply altering the size of the portal of radiation or appropriately positioning the animal in relation to the x-ray beam. During our study there was no difficulty in consistently irradiating the apical area of the heart. Furthermore, one may be able to vary the degree of myocardial fibrosis or necrosis by altering the total dose of irradiation given or by changing the rate at which roentgens are delivered.

Summary. The radiation effects of 2,500 to 20,000 roentgens on the canine myocardium have been studied. A collimated x-ray beam, 2.54 cm in diameter, was delivered to the heart of 12 dogs at a rate of 210 roentgens per minute. A localized area of myocardial necrosis was evident by the 7th to 10th post-irradiation day in the hearts which received a dose of 20,000 roentgens. Evolution of the myocardial lesion was documented electrocardiographically, and by pathologic examination. Microscopically, the cardiac necrosis was strikingly similar to vascular infarction in the human myocardium. The use of this radiation technique for experimental production of localized myocardial necrosis is discussed.

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Received February 4, 1963. P.S.E.B.M., 1963, v112.

Studies on Growth of Eaton's Agent in Tissue Culture.* (28206)

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Evidence that Eaton's atypical pneumonia agent is a pleuropneumonia-like organism (PPLO)(1-3) provides impetus for further investigation of biologic properties which have been difficult to determine for lack of convenient and sufficiently reproducible quantitative methods. After direct demonstration of the agent was accomplished in tissue culture(2), attention was given to possible use of this system to circumvent assay problems encountered by earlier investigators(4,5,6). This report concerns a method of quantitation based upon enumeration of Eaton PPLO colonies visualized in tissue cultures. Applications of this technique are presented in elucidating growth of the PPLO and growth inhibition by antibiotics and antisera.

Materials and methods. The Mac strain of Eaton's agent, obtained originally from Dr. Chien Liu, was passaged 8 times in chick embryos, and once in monkey kidney cell cul-

* This investigation was conducted under the sponsorship of Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported by Office of The Surgeon General, Dept. of the Army.