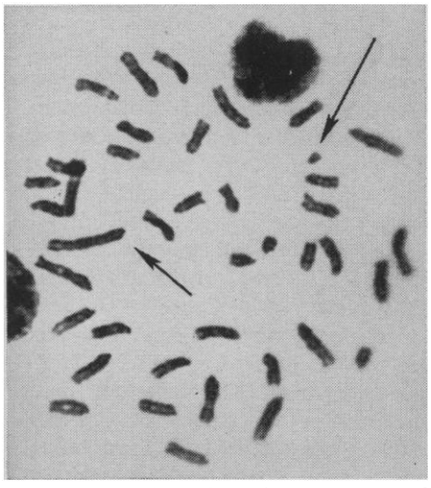

Fig. 1. Metaphase from bone marrow of LAf_1 mouse x-irradiated (500 rad, dose rate 30 rad/min) 72 weeks previously. Note the abnormally large and minute chromosomes indicated by arrows. Twenty percent of the marrow cells examined were of this type.

dose rate could similarly be due to an intracellular recovery process, perhaps involving restitution and repair of radiation-induced chromosome breaks comparable to that observed by Wolff (*9*) in plant cells. This recovery process would presumably be analogous to that described by Elkind and Sutton (*10*) in studies of the viability of hamster cells in tissue culture after fractionated radiation. In their work, however, irradiation was completed within one mitotic cycle, while in our experiments with low dose rates, numerous generations of cells were involved. Hence, it is possible that additional recovery mechanisms, both intracellular and extracellular, might operate in our system.

It is also possible that both the high and low dose rates may have produced the same amount of non-recoverable chromosome damage, and that cell selection rather than intracellular recovery mechanisms are responsible for the observed difference in persistent chromosome changes. The extensive destruction of marrow cells which occurs at the high dose rate, producing an aplastic marrow for a brief period, could provide the opportunity and "space" for a few stem cells, with radiation-induced chromosome changes conferring a slight growth advantage, to repopulate the marrow with recognizable clones. Cells with similar changes produced by low dose rate radiation, which does not deplete the marrow, might not have a sufficient selective advantage to permit them to overgrow an already populated marrow and, hence, they would continue to survive as only a small proportion of the marrow cells. The continued persistence of large clones of abnormal cells, without concomitant hematological disorders, in animals given a high dose rate indicates that radiation-induced chromosome abnormalities do not necessarily confer either a marked selective advantage or disadvantage on cells bearing them (*6*).

Although it might be expected that Co^{60} γ-radiation would produce fewer breaks per rad than x-radiation on the basis of the well-known difference in the relative biological effectiveness between these radiations, this would not, by any means, explain the marked difference between the effects of low and high dose rates observed in the present study.

The present study permits further speculation on the relationship of radiation-induced chromosome changes to leukemogenesis. Data from both mice and humans indicate that there is no uniform correlation between demonstrable chromosome changes produced by radiation at high dose rates and the subsequent development of leukemia (*6*). Whether the incidence of radiation-induced leukemia in mice exposed to γ-radiation at a low dose rate would parallel the present chromosome findings remains to be determined. Single acute exposure at a high dose rate, however, may be lethal for a larger number of marrow stem cells than is radiation given at a low dose rate, thus possibly eliminating many potentially leukemic cells from the "pool"—the so-called "therapeutic" effect of high radiation dose on leukemia incidence (*11*). If leukemogenesis is related to the total number of point mutations produced, it is conceivable that the frequency of point mutations could be the same in all three groups in the present study and that the incidence of leukemia might actually be highest in the group exposed to radiation at the low dose rate, as a result of accumulation of such radiation-induced point mutations with a minimum of cell killing. Observations on leukemia incidence in mice exposed to low dose rate γ-radiation are now being made.

The relationship between chromosome aberrations and the occurrence of other late pathological effects of irradiation, such as solid-tissue tumors, nephrosclerosis, and other lesions resulting in shortened life span, is even less clear. Data are not available on chromosomes from the organs involved, and extrapolation to these tissues from the effects of dose rate on bone marrow cells might well be erroneous, because of the great differences in mitotic rates and cell turnover. It would be of interest to assess the importance of chromosome damage in the production of late radiation effects in organs such as the liver and kidney (*12*).

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Reserpine: Its Effect on Silver-Stained Structures of the Heart

Abstract. *The administration of reserpine to dogs in doses sufficient to deplete myocardial catecholamines resulted in alterations in the affinity of the heart for silver stain. Most noticeably affected was the "perimysial plexus."*

Silver-stained preparations of heart tissue from many species reveal a plentiful mesh of fine and course tortuous structures which appear to envelope cardiac muscle cells. Some evidence indicates that these structures constitute

a portion of the innervation of the heart which has been referred to as a perimuscular or perimysial plexus (*1*).

The catecholamine content of the heart has been related to its sympathetic motor innervation (*2*). Since the administration of reserpine will cause a depletion in the myocardial stores of catecholamines, it seemed pertinent to determine whether this drug might affect the affinity of the tissues for the silver stain. Such an alteration would provide evidence for the relation of these silver-stained structures to the innervation and might provide a basis for the determination of what constituents are participating in the deposition of the silver salts in the tissue.

The hearts of eight dogs were studied. Three dogs were untreated. Reserpine was administered by intramuscular injection to five dogs. Two dogs were given 2.5 mg/kg 24 hours before study; one was given 2.5 mg/kg 15 days before study; one received 0.1 mg/kg 24 hours before study, and one dog received 0.85 mg/kg in three doses 24 hours before study. At the time of study the dogs were anesthetized with pentobarbital or secobarbital sodium, the hearts were excised, and biopsies were performed. The tissue thus obtained was frozen at the temperature of dry ice and subsequently analyzed for catecholamine content by a fluorometric method (*3*). The hearts of the dogs were then fixed in Zenker's solution without acetic acid. After at least 72 hours fixation, blocks of tissue for sectioning were taken from the right and left atria, ventricles, papillary muscles, and the septum. The blocks of tissue were embedded by the celloidin-paraffin method and the sections were cut serially. Sections were cut to cover 20 to 40 slides. The sections on alternate slides were stained by the Gomori trichrome method; those on the slides between were processed according to the silver-reduction procedure of Nasser and Shanklin (*4*), and then counterstained by the trichrome method.

The amount of reserpine administered was effective in producing depletion in myocardial catecholamines. Values from three normal animals averaged $0.67 \pm 0.10 \mu g/g$ of tissue, whereas values from ventricular biopsies from the five reserpinized animals averaged 0.03 ± 0.02 $\mu g/g$.

Sections of myocardium from untreated, normal dogs studied by this method showed certain characteristics (Fig. 1). The muscle showed distinct cross striations. When viewed in longitudinal section, dense sinuous black structures were interposed between the muscle bundles. These appeared to give rise to finer, tortuous structures which were wrapped around the muscle strands. These fine fibrillar structures constitute the perimysial plexus.

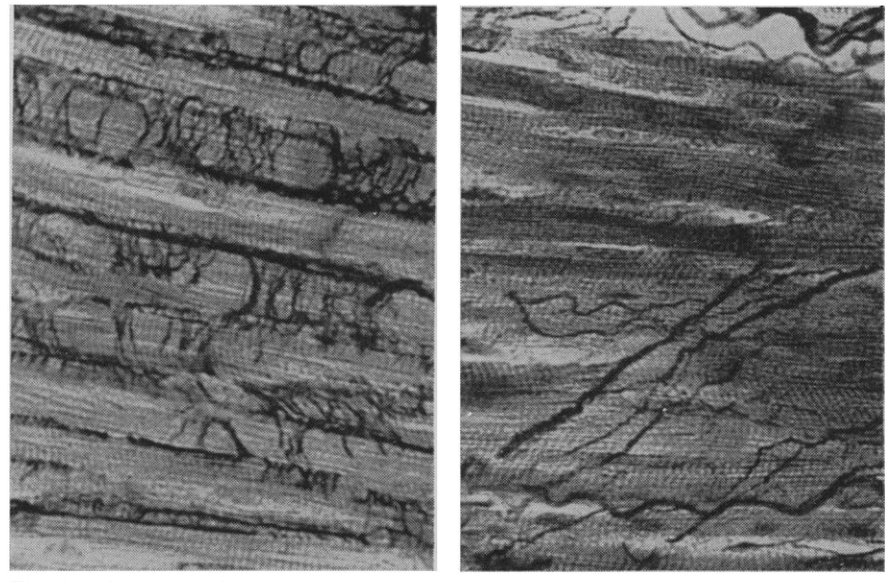

Fig. 1 (left). Photomicrograph illustrating the structure of the silver-stained perimysial plexus in the left ventricle of a normal dog (about × 280). The appearance of this region is representative of the appearance of the entire myocardium. Fig. 2 (right). Photomicrograph illustrating extensive reduction in perimysial plexus in the left ventricle of reserpinized dog (about × 280). A similar reduction was present in all regions of the myocardium.

Study of the hearts from animals treated with reserpine revealed significant changes in all regions sampled (Fig. 2). The silver-stained structures, particularly the perimysial plexus, in the dogs receiving 0.85 or 2.5 mg/kg, were reduced extensively to faint outlines with scattered granules; the larger structures, singly and in fascicles, were granular and pale. Many fascicles were argyrophilic and their margins were beaded; others showed only the blue color of collagen. Changes in the appearance of tissue from the dog which received 0.1 mg/kg were less striking.

Other studies (*5*) have shown that vagotomy is followed by a similar modification in the appearance of these silver-stained structures. There was extensive retrogression in the larger silver-stained fascicles. Much of the perimysial plexus disappeared, leaving argyrophilic granular residues. However, the catecholamine contents of chronically vagotomized heart are not extensively modified. These observations would seem to indicate that depletion of catecholamine may be one of several mechanisms which could alter the appearance of the silver-stained tissues of the heart. Reserpine is known to affect other tissue constituents which could participate in the reaction (*6*). These studies do not prove that the silver-stained tissue is in fact nerve tissue. However, the alteration of the perimysial plexus by interventions which influence the innervation of the heart suggests that the plexus might be part of the innervation apparatus (*7*).

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