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A Review of the Histopathological Effects of Ionizing Radiation on the Pacific Oyster, *Crassostrea gigas*

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It has long been known that ionizing radiation causes deleterious effects in mammalian cells and tissues (Bergonie and Tribondeau, 1906). During the past 30 years, the mechanisms responsible for the various mammalian radiation syndromes of cellular and tissue degeneration have been elucidated (Bacq and Alexander, 1961; Patt and Quastler, 1963; Fabrikant, 1972).

Excepting studies of the effects on planarian regeneration, and those associated with "sterile male" techniques for biological control of insects, there have been few studies concerned with the pathological effects of ionizing radiation on metazoan invertebrates. During the early 1970's, we published a series of papers on the histopathological effects of ionizing radiation on the Pacific oyster, *Crassostrea gigas* (Mix and Sparks, 1970; Mix and Sparks, 1971a, b; Mix, 1972). Unfortunately, at that time little was known about normal cell renewal systems (CRS) of bivalve mollusks and thus, we were forced to interpret many of the results of those studies without adequate knowledge of these systems. Since that time, my graduate students and I have corroborated some of the assumptions and tentative descriptions of bivalve CRS made during those studies.

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Radiation effects at the tissue, organ, and organism level are most easily explained by describing the effects on normal CRS. Tissues with cell turnover depend for their integrity on continued cell production, and perturbations of the CRS can be brought about, perhaps most dramatically, by exposure to ionizing radiation. The effects of irradiation on most CRS can be reduced to a simple basic scheme; initially, there is impaired cell production with little change in the rate of cell loss, which leads to cell depletion; the degree of depletion depends on the extent to which cell production is impaired. Thus, radiation causes aplastic cytopenia or, if a cell population vanishes completely, acytosis (Patt and Quastler, 1963). More or less complete restoration of the system may be possible if the organism survives until stem cell proliferation can be successfully resumed. The effects of irradiating certain oyster tissues and cells can be explained within this scheme, although it is difficult to make precise analyses because of the paucity of knowledge about cell kinetics in these animals.

Basically, oysters were irradiated with various doses of gamma radiation (0, 1, 10, 20, 50, 100, 200, and 400 krads), maintained in a continuous flow-through seawater system 9°-15°C (ambient temperatures) and sampled for 90 days during the first study and 180 days during a second study in which all oysters were irradiated with 75 krads. All oysters were prepared for histological examination and over 1,000 animals were analyzed during these studies (see Mix and Sparks, 1970; Mix and Sparks, 1971a, b; and Mix, 1972 for additional details).

A brief summary of the most significant results is included below.

1) All oysters irradiated with 200 and 400 krads died within 9 days while oysters receiving 0-100 krads did not experience mortality within the 90-day experimental period (Mix and Sparks, 1970).

2) Oysters irradiated with 75 krads died approximately 180 days post-irradiation (Mix, 1972).

3) Chronic degeneration, thought to be caused by mitotic inhibition and/or complete failure of cellular or subcellular repair mechanisms, was evident in all tissues dependent on CRS for their integrity (Fig. 1A-H). Quantitative data indicated that mitotic cell division had ceased in the gut by 8 days in oysters irradiated with 10 or more krads and did not resume during the experimental period. As a result of mitotic inhibition, there was a continuous decline in the epithelial cell population of this tissue. It was felt that similar phenomena accounted for the chronic degeneration of other tissues (Mix, 1972).

4) The mortality that occurred by 180 days after irradiation with 75 krads was presumably related to the failure of CRS to restore depleted cell populations and was thought to be particularly important with regard to the gills and digestive tubules. The cell populations in these two tissues by 180 days were either greatly diminished (gills) or had completely disappeared (digestive tubules) and thus, the actual cause of death was probably starvation.

5) Restoration of injured and destroyed cell populations was observed in only one tissue, the digestive tubules, in oysters irradiated with sublethal doses. Desquamated tubules were repopulated after rapid proliferation of unusually large crypt cells (Mix and Sparks, 1971a). It is interesting to note that these cells did not differentiate; it is not known if this was due to low water temperatures or perhaps the absence of a "maturing factor." There

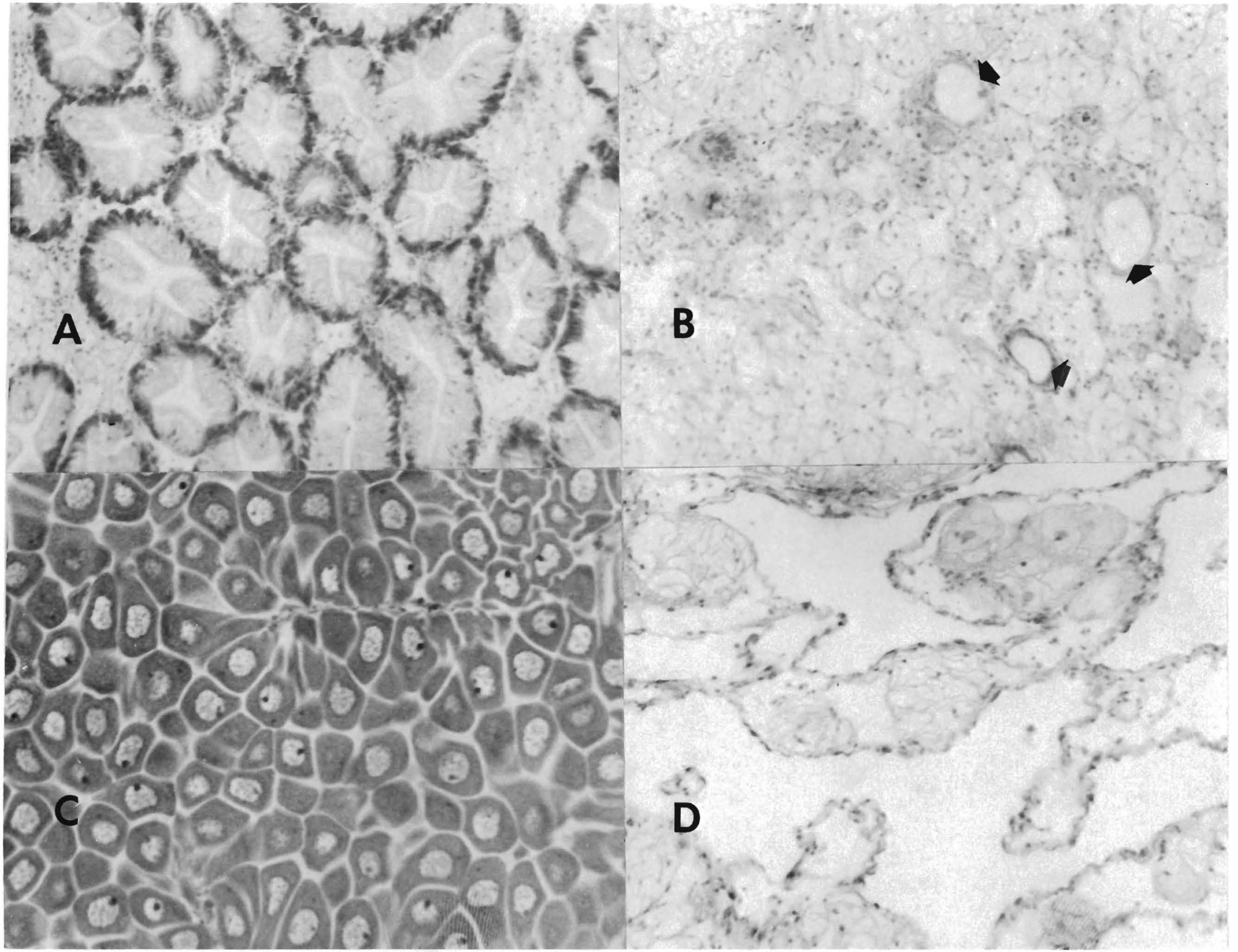

Figure 1 (A-D).—Normal tissues of *Crassostrea gigas* 180 days after sham irradiation and tissues 180 days after irradiation with 75 krads (128×). A. Normal digestive tubules. B. Digestive tubule area after irradiation; arrows indicate denuded tubules which were not repopulated. C. Normal gonad containing mature ova. D. Irradiated gonad. All oysters were maintained in the same system.

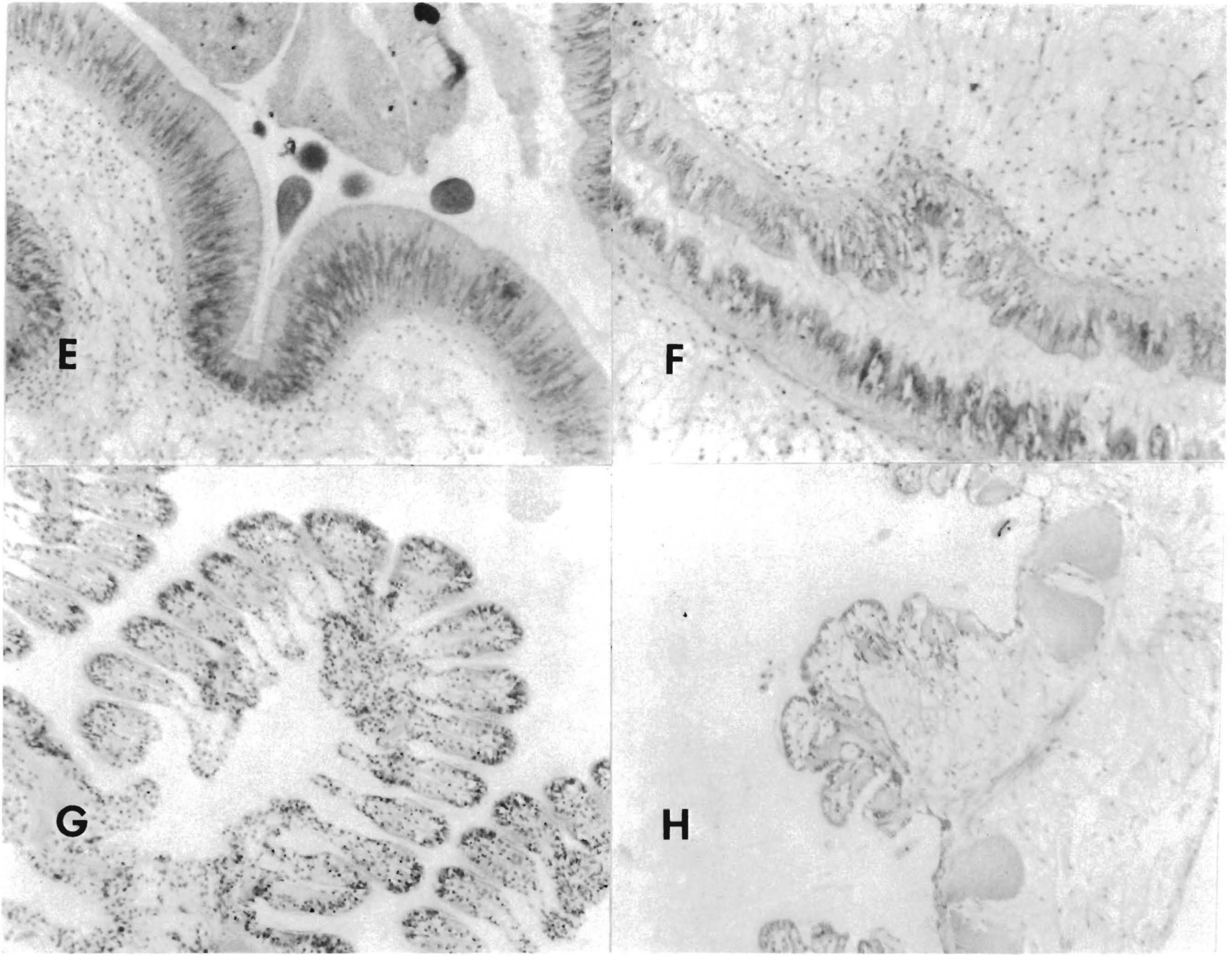

Figure 1 (E-H).—Normal tissues of *Crassostrea gigas* 180 days after sham irradiation and tissues 180 days after irradiation with 75 krads (128×). E. Normal gut containing unassimilated food. F. Irradiated gut. G. Normal gill. H. Irradiated gill. All oysters were maintained in the same system.

was no evidence to indicate that tubules repopulated by the undifferentiated crypt cells ever became functional.

6) Doses as low as one krad caused degeneration of the gonad in some oysters and doses in excess of one krad effectively sterilized all oysters. It appeared that sterilization was permanent as evidenced by the formation of scar-like tissue in the gonad (Mix and Sparks, 1971b).

The effects of ionizing radiation on *C. gigas* can be explained and interpreted, in part, by analyzing the effects on normal CRS. The evidence suggests that depletion of essential cell populations was directly or indirectly responsible for death of the oyster which occurred 5-6 months after irradiation with 75 krads.

From our studies, certain conclusions can be made about tissue sensitivity and restoration of depopulated tissues in *C. gigas*. Since cell depletion rates are directly related to cell turnover times, the order of radiosensitivity in oyster tissues is probably (most to least sensitive): Gonad, gill, digestive tubules, mantle, and other digestive tissues. Leydig, muscle, and nerve cells are quite resistant, which is consistent with their apparent inability to proliferate. *Crassostrea gigas* gills appear to possess certain cellular mechanisms, first described in the mammalian gut, which can partially compensate for the accelerated rate of cell loss caused by ionizing radiation; these include a delay in the rate of cell loss by extension of the transient time spent on the gill filament and hypertrophy of remaining cells which prevents denudation of the filament. Digestive tubule cells may have exhibited a mechanism associated with accelerated cell repopulation in some mammalian tissues —inhibition of differentiation until the cell population of a tissue is restored.

It seems apparent that the LD_{50} concept so widely used in radiation biology has little practical value in studies on metazoan invertebrates. It is evident that because of slow cell turnover times, radiation effects may not manifest themselves until months or even years after irradiation. The old idea that invertebrates are resistant to radiation can no longer be accepted unequivocally. Careful studies utilizing low doses of radiation and extended time periods are clearly necessary to establish the exact sensitivity of any particular invertebrate.

There are many unanswered questions which will hopefully be the subject of future investigations. Among them are these:

1) Do bivalve mollusks possess subcellular repair mechanisms or were the digestive tubule cells involved in repopulation simply survivors and not "repaired cells?"

2) What are the kinetics of tissue repair?

3) What are the effects of temperature on subcellular repair and cell and tissue renewal?

4) How does temperature affect the rate of tissue degeneration and subsequent death of the organism?

5) Why did the digestive tubule cells involved in tissue repopulation fail to differentiate?

6) What doses can cause more subtle negative biological effects (mutations, neoplasia, life-span shortening)?

7) How do leucocytes fit in; are they involved in tissue repair; what is the effect of irradiation on leucocyte cell renewal?

Finally, it is obvious that in order to answer many of these questions it will be necessary to utilize autoradiographic techniques. Unfortunately, attempts to apply such methods on *C. gigas* have failed (Mix, 1972; Mix and Tomasovic, 1973) and thus, it may be necessary to select a different species for use as a model in understanding the effects of ionizing radiation on bivalve mollusks.

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