

AN ABSTRACT OF THE THESIS OF

Steve Ray Trenholm for the degree of Master of Science

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Title: EFFECTS OF X- AND GAMMA IRRADIATION ON THE JUVENILE PACIFIC

OYSTER, CRASSOSTREA GIGAS

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Abstract approved: \_\_\_\_\_

Michael C. Mix

Two separate studies were conducted during this research project. Oysters were irradiated with 500 and 1000 rads and 5, 10, 20, 50, 75, 100, 150, and 200 krads in the first study in order to determine the effects of ionizing radiation on survival and growth rates. Two periods of high mortality were noted; the first occurred from 2 to 7 days postirradiation in oysters receiving 75 to 200 krads and was associated with an "acute lethal tissue degenerative syndrome." The second mortality period occurred from 40 to 60 days postirradiation in oysters exposed to 10 krads or more and was caused by a "lethal tissue degenerative syndrome." The LD-50 dose was found to be a complex function of time from the moment of irradiation until approximately 80 days postirradiation. The 238-day LD-50 value was 16.5 krads.

The mean wet weight of oysters exposed to 20 krads was significantly less than that of the controls from 167 to 238 days postirradiation. Analysis of the results suggest a dose dependent wet-weight relationship in the 5 and 10-krad oysters; they did not

weigh significantly less than the controls. Although not statistically significant, the mean wet weights of oysters exposed to 500 and 1000 rads exceeded that of the controls from 43 to 238 days postirradiation.

In the second study, oysters were irradiated with 200 R, 600 R, 1000 R, 5 kR (X irradiation), and 8, 16, and 40 krads (gamma irradiation). The purposes of the second study were to analyze histopathologically, the degenerative syndromes and subsequent tissue repair processes in the stomach, gut, collecting ducts, and digestive tubules. Degenerative changes were seen only in the digestive tubules of 5- and 8-kR oysters while in oysters exposed to 16 and 40 krads, degenerative changes coincided with the lethal tissue degenerative syndrome noted in the survival-wet weight study. A tissue regeneration sequence was observed in the stomach, gut, collecting ducts, and digestive tubules of most oysters exposed to 16 krads and in a smaller number of oysters exposed to 40 krads. Tissue regeneration was first observed in the digestive tubules and subsequently in the stomach, gut, and collecting ducts. Repopulation of the digestive tubules involved reepithelialization of the tubule with large, undifferentiated crypt cells followed by their differentiation into secretory and absorptive cells. Tissue recovery in the stomach, gut, and collecting ducts was initiated by islands of small basophilic cells not previously described in these tissues. Rapid mitotic proliferation of these cells and their subsequent differentiation into basal epithelial cells, resulted in the reepithelialization and eventual recovery of these tissues.

Effects of X- and Gamma Irradiation on the Juvenile  
Pacific Oyster, Crassostrea gigas

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Effects of X- and Gamma Irradiation on the Juvenile  
Pacific Oyster, Crassostrea gigas

INTRODUCTION

The long-term effects of ionizing radiation on most marine organisms are virtually unknown. Lengthy studies of many of these organisms are difficult to conduct because of the problems involved in long-term maintenance of laboratory populations. Moreover, it seems likely that external environmental factors may significantly influence the radiation response of many marine organisms. For example, since most aquatic estuarine organisms are euryhaline poikilotherms, their responses to ionizing radiation may differ as the temperature and salinity of their environment varies. Furthermore, some intertidal organisms (bivalve mollusks) are facultative anaerobes and as a result, the presence or absence of oxygen in their tissues may influence their radiation response.

Because of earlier studies in which many aquatic invertebrates appeared unaffected by doses of ionizing radiation that would injure or kill most mammals, there has been a tendency to describe invertebrates as being generally radioresistant. It is possible that long-term studies will show that some aquatic invertebrates are not as radioresistant as previously thought. It seems likely that low metabolic and/or slow cell turnover rates may retard the appearance of radiation damage which is then not manifested for months or even years after an acute exposure. Thus, while acute effects may be relatively unimportant, long-term effects such as life-shortening, neoplasia, or a change in the genetic constitution of the population may be

paramount in these organisms.

Very little information is available concerning postirradiation (PI) survival of aquatic mollusks. Most radiation studies have shown both PI survival and radiosensitivity to be functions of time. The most common measure of radiosensitivity is the LD-50 dose. That is, the radiation dose required to kill 50 percent of a group of organisms within a specified time period. The 30-day LD-50 dose has become the standard measure of radiosensitivity in mammals because most acute mammalian radiation syndromes occur prior to 30 days. However, for invertebrates and lower vertebrates the LD 50 is probably not a valid measurement of radiosensitivity because the acute radiation syndromes may not occur by 30 days PI. Perhaps more accurate measures of radiosensitivity may be achieved by determining the LD-50 dose after the development of the acute radiation syndromes. Before radiation sensitivity can be accurately determined in invertebrates, it is first necessary to complete long-term survival studies and histopathological descriptions of the acute radiation syndromes. To date, neither long-term survival studies nor histopathological work has been conducted on most marine organisms.

Hargis et al. (1957) compared the PI survival of male and female oyster drills, Urosalpinx cinerea, at 30 and 60 days following exposures ranging from 3 to 48 kR. They observed a sharp increase in the number of mortalities in both males and females from 30 days until the experiment was terminated 69 days PI. In a later study (Rice and Baptist, 1971), the data for the oyster drill experiment was described in terms of radiosensitivity.

It was noted that the LD-50 dose increased from 30 to 60 days PI for both male and female snails. Furthermore, males were found to be more radiosensitive than females at both 30 and 60 days PI.

Perlowagora-Szummlawicz (1964) studied survival, growth, and fecundity of snails (Australorbis glabratus) that developed from eggs exposed to x-rays (2-15 kR). Hatching was delayed in the irradiated eggs and there was also a subsequent delay in the rate of development in the small snails. In addition, snails hatched from irradiated eggs were reported to show signs of decreased survival and growth, and to experience a delay in the onset of sexual maturity. It was also observed that an increase in radiation dose resulted not only in a progressive decrease of egg hatches, but also in a progressive decrease in survival among hatched snails. Radiosensitivity for the snails hatched from irradiated eggs was later reported (Rice and Baptist, 1971) to decrease as time PI increased. For example, the LD-50 dose at 30 days was 23.8 kR while at 90 days it was 13.8 kR.

Studies of the clam, Mercenaria mercenaria, and the oyster, Crassostrea virginica by Price (1965) revealed that in both species, there were large increases in the number of mortalities from 30 to 90 days PI following gamma exposures of 23 to 187 kR. The 30-day LD-50 doses were later determined to be 115 kR for the clam and 100 kR for the oyster (Rice and Baptist, 1971). The LD-50 doses at 90 days PI were found to be drastically reduced for both the clam (67 kR) and the oyster (< 10 kR). A similar increase in radiosensitivity was noted in the mud snail, Nassarius obsoletus, following gamma irradiation (White and Angelovic, 1966). They reported a 15-day LD-50 dose of 51.5 kRads

and a 50-day LD-50 dose of 14 krads.

Baptist et al. (1976) exposed the clam, M. mercenaria, and the scallop, Argopecten irradians, to chronic gamma radiation and studied the survival patterns and growth rates. For clams, they observed a decrease in survival only in the highest exposure group (37 rads per hour) during the 14-month experimental period. They noted no decrease in survival of the scallops during the 84 days they were observed. They also found that clams exposed to low gamma radiation levels increased more rapidly in weight but not in length than the controls. Only at the highest exposure rate did they observe a decreased growth rate for clams. They also noted that continuous gamma irradiation (cumulative dose of 71.7 krads) appeared to have no effect on growth of the scallops during the 84 days they were observed.

The effects of chronic radiation on both laboratory and natural populations of the aquatic snail, Physa heterostrophia, were conducted by Cooley and Miller (1971) and by Cooley (1973). In the laboratory studies, snails were exposed to continuous dose rates of 1, 10, and 25 rads per hour. They reported that a dose rate of 25 rads per hour eliminated reproduction and led to extinction of the population within one generation. Their data also supported the contention that a dose rate of 10 rads per hour led to extinction of the population in two generations. They also noted that in populations exposed to 1 rad per hour, there were indications of reduced fecundity. Histopathological examinations revealed partial atrophy of the ovotestis in the 10-krad snails and total atrophy in the 25-krad snails. In further studies of natural populations of snails exposed to chronic

environmental radiation (0.65 rads per day), Cooley (1973) found that when compared to a control population, the frequency of egg capsule production in the irradiated population was reduced. However, there was an increased number of eggs per capsule in the irradiated population which brought the total number of eggs produced per snail in the irradiated population to approximately the number produced in the control populations. He suggested that natural selection brought about the increased number of eggs produced per capsule in the irradiated snails as a compensating mechanism for the reduced capsule production.

Studies of the histopathological effects of ionizing radiation on the adult Pacific oyster, Crassostrea gigas, were conducted by Mix and Sparks (1970) and by Mix (1972). In the first study, oysters were exposed to doses of gamma radiation ranging from 1 to 400 krads and observed histopathologically for 90 days. In the second study, oysters were exposed to 75 krads and then observed histopathologically for 120 days. The degenerative syndromes observed in these studies were described by Mix (1972). An acute lethal syndrome was observed in oysters exposed to 200 and 400 krads and a chronic syndrome occurred in oysters exposed to 1 to 100 krads. All oysters except one in the groups affected by the acute lethal syndrome died by 11 days PI. Histopathological examination revealed that the acute syndrome consisted of numerous cellular and tissue alterations prior to cell fragmentation or subsequent cell death. Moreover, Mix (1972) noted that the chronic degenerative syndrome consisted first of an acute tissue response that was observed for 1 to 9 days PI and a chronic

tissue degenerative phase which occurred from 10 to 59 days PI in the first experiment and from 10 to 180 days PI in the second experiment. No significant mortalities were observed in oysters receiving doses of 1 to 100 krads in the first study. In the second study no mortalities were noted in the 75-krad oysters prior to 120 days, but 10 of the 12 remaining oysters died between 120 and 180 days PI.

Repair of digestive tubule tissue in the Pacific oyster following a 20-krad gamma exposure was described histopathologically by Mix and Sparks (1971). They noted that during the degenerative phase, the normal epithelial cells (crypt or stem cells and secretory and absorptive cells) were sloughed, leaving the digestive tubules denuded. Repopulation of the digestive tubules began with the formation of cell nests or epithelial islands of crypt cells. Differentiation of crypt cells into secretory and absorptive cells was not observed to occur during the 90 days of the study.

The earliest hypothesis to explain the relationship between radiation and cell renewal processes was proposed by Bergonié and Tribondeau (1906). They stated that x-ray sensitivity of a cell varied directly with the rate of proliferation and the number of future divisions and inversely with the degree of morphological and functional differentiation.

It has been suggested that PI survival of organisms depends on the survival and integrity of cell renewal systems (Patt and Quastler, 1963). In cell renewal systems (CRS) of mature animals, an equilibrium is maintained between the rate of cell production and the rate of cell loss. Cell renewal systems therefore depend upon continued cell

production for their integrity. However, cell production is notoriously susceptible to perturbations by ionizing radiation (Patt and Quastler, 1963). In other words, if cell production is retarded by radiation, depletion of the stem cell population may occur. If enough stem cells are lost, the ability of the organism to recover from radiation injury may be lost or severely impaired.

In the present research, two studies were conducted. One was concerned with determining long-term survival and changes in mean wet weight following exposure to varying doses of gamma radiation. The purpose of the second study was to analyze histopathological changes in the digestive tissues following exposure to different doses of x- and gamma radiation. Objectives of these studies were: (1) to analyze the long-term PI survival patterns of juvenile Pacific oysters; (2) to determine long-term PI growth patterns for juvenile Pacific oysters; (3) to correlate long-term histopathological effects with survival and growth patterns. These studies were designed to answer the following questions: (a) Are oysters radioresistant; or is radiation damage masked or delayed because of low metabolic rates and/or slow cell turnover? (b) Does complete repopulation of digestive tissues with normal cells follow radiation-induced degenerative changes? If so, how long after irradiation and at what doses? (c) Are the PI histopathological changes in juvenile Pacific oysters similar to those observed in the adult oysters (Mix and Sparks, 1970; Mix, 1972)? (d) Can PI growth patterns be used in oysters as indicators of radiosensitivity?



## MATERIALS AND METHODS

### Animal Collection and Maintenance

Pacific oysters, Crassostrea gigas, were selected for these studies for several reasons. It was possible to acquire genetically similar stock, maintenance facilities were available at Yaquina Bay, Oregon, and information about the effects of radiation on this animal was available from previous studies.

One thousand six-month-old cultchless (not attached to mother shell) Pacific oysters were purchased from International Shellfish Enterprises, Moss Landing, California for use in the survival/body (wet) weight experiment. They were acclimatized at Oregon Oyster Company, Yaquina Bay, from 23 April 1976 until 9 July 1976. During this time they were maintained in a 2 feet by 3 feet by 10 inch polyvinyl chloride (PVC) box which was perforated to allow free flow through of water. That box was suspended from a floating dock and held approximately 4 feet beneath the surface of the water. Oysters were checked periodically during the acclimatization period for the presence of accumulated silt and predators.

For the histology studies, 18,000 eight-week-old cultchless Pacific oyster spat were obtained from Pigeon Point Research Center, Pescadero, California. They were acclimatized at Oregon Oyster Company from 11 November 1975 until 17 April 1976. The oysters were maintained in a perforated PVC box suspended at a depth of 8 feet and siltation mud was periodically removed to prevent suffocation of the

juvenile oysters during this period.

### Survival and Mean Wet Weight Studies

Prior to the survival/wet weight experiment, 216 of the 9-month old oysters were divided into 12 groups each of 18 animals and then brought to Corvallis. During the trip from Yaquina Bay to Corvallis they were kept in a plastic bucket containing seawater and maintained at the same temperature as the bay by using ice packs.

Oysters in 10 of the 12 groups were exposed to gamma irradiation (1.17 and 1.33 MeV) in the Cobalt 60 food/research irradiator located at the OSU Radiation Center. Groups of oysters were irradiated with either 500 or 1000 rads at a dose rate of 296 rads per minute or 5, 10, 20, 50, 75, 100, 150, and 200 krad at a dose rate of 1760 rads per minute. Dose rate fields for the Cobalt irradiator were calculated using calibrated thermoluminescent dosimeter chips (Harmon, 1976). During irradiation, oysters were placed in a single layer around the outside of a 11 mm diameter lucite dish, thus, all oysters were in a relatively uniform gamma field. Two control groups, both sham irradiated, were included in this study. One was sham irradiated for 12 minutes while the other group received 114 minutes which corresponded to the exposure time of the highest dose group.

Immediately after irradiation, all groups were placed in a seawater aquarium maintained at the temperature of the bay. After 3 days all oysters except those in the 200-krad group were transported back to Yaquina Bay where each group was placed in an

individual compartment of an oyster growout tray and maintained at a depth of 4 feet. Because of the early acute mortality pattern in the 200-krad group, those oysters were kept in the aquarium and observed daily.

Each of the 12 groups of oysters in this study were monitored for mortalities on the second, third, fifth, and seventh day PI and then weekly for 14 weeks. Beginning with week 16, they were checked monthly until 9 months PI. An oyster was considered dead when the adductor muscle could no longer keep the two valves closed. Using the mortality data, a percent survival vs days PI plot was made. Mean survival times were also calculated for each dose group and plotted as a function of time. In addition, the LD-50 values were calculated from the survival curve by the midpoint estimate method of Reed and Muench (1938) and plotted against days PI.

Just prior to irradiation, each group of oysters was weighed on a Mettler top-loading balance and the mean weight was calculated by dividing total group weight by the number of oysters in the group. Each group was then weighed every 2 weeks for the first 4 months and once at 5 months PI with an Ohaus portable triple beam balance. Each oyster was scrubbed with a stiff bristle nylon brush to remove algae and mud from the shell before they were weighed. They were then rinsed, blotted dry, and weighed. Four times during the scrubbing process an oyster was accidentally dropped into the bay and lost. That occurred on week 8, when one oyster each was lost from a control group and from the 500-rad group, and on week 25 when one animal was lost from both a control group and the 5-krad group. Starting with

the sixth month oysters from the surviving groups were taken monthly to the OSU Marine Science Center, cleaned and weighed individually on a top-loading Mettler balance. Individual measurements permitted calculation of the standard error in each group.

The mean wet weight of each group was plotted as a function of time from just prior to irradiation to 238 days PI. Growth rates were also calculated from 98 to 238 days PI for the 10 krad, 20 krad, and control groups.

### Histopathology Studies

For the histopathology studies, 5500 of the 7-month-old oysters were divided into 10 groups of 550 animals each. Nine of the groups were brought to Corvallis on 17 April 1976 and eight were irradiated with various doses of radiation while the other group served as an in-bay control group. During the trip from Yaquina Bay to Corvallis and back, oysters were maintained in the manner described previously.

A General Electric Maxitron 300, deep therapy x-ray unit, located at the OSU Radiation Center was used to irradiate five of the eight groups. The operating parameters of exposure were 300 KVP, 20 mA, and a half-value thickness of 2.0 mm Cu (temperature 20°C, pressure 775 mm Hg, relative humidity 48%). The exposure rate was determined by utilizing calibrated Victoreen Thimble Chamber Dosimeters. Single groups were exposed to 200 and 600 R given at a rate of 50 R per minute and 1000, 2000, and 5000 R given at a rate of 200 R per minute. During irradiation, oysters were placed in a single layer on a 20 cm diameter lucite dish mounted on a turntable

which allowed for a uniform exposure distribution. The remaining three groups were exposed to gamma irradiation in the Cobalt 60 food/research irradiator located at the OSU Radiation Center. The dosimetry and irradiation procedures were the same as described previously. Oysters in the three groups were irradiated with 8, 16, and 40 krads given at a dose rate of 1854 rads per minute.

Because it is composed of primarily low atomic weight materials, the oyster shell would be expected to cause little or no attenuation of the x- or gamma-ray field. Mix (1970) has shown this to be the case for 250 KVP x-rays.

Histopathological examinations of prepared slides were made of oysters sacrificed periodically between 6 days and 321 days PI. For the first 10 weeks, 4 oysters per week were sampled from each irradiated group. From 10 weeks to 20 weeks PI, 4 oysters from each group were sampled every other week. Beginning with week 20, each group was sampled monthly and every other sample consisted of 20 oysters per group. Only four oysters were taken from each group during the other monthly sampling.

The dorsal-ventral length of each sampled oyster was measured and the mean shell length calculated for each group. Oysters were then shucked (removed from the shell) and fixed for at least 24 hours in Davidson's fixative (formalin, seawater, glycerin, ethanol, and acetic acid added just prior to use). They were then dehydrated through a graded series of ethanol, cleared with xylene, and embedded in Paraplast. Finally, 6 micron sections were stained with neutral Harris' hematoxylin and eosin. The histopathological condition of the

stomach, gut, collecting ducts, and digestive tubules was then examined using a Zeiss Standard research light microscope.

## RESULTS

### Postirradiation Survival

A rather complex survival pattern was discerned following irradiation of juvenile Pacific oysters (Figure 1). Postirradiation survival of oysters exposed to 50 krad or more was dose independent; that is, all groups exposed to doses of 50 krad or more experienced 100% mortality within 3 months. Groups that received doses of less than 50 krad had PI survival patterns which appeared to be dose dependent (i.e., the greater the dose, the greater the number of mortalities).

There were two time periods when numerous mortalities were observed (Figure 1). The first of the mortality periods was roughly correlated with the occurrence of an acute lethal (AL) tissue degenerative syndrome described by Mix and Sparks (1970). In the present experiment, the AL syndrome occurred from 2 to 7 days PI and was associated with mortality in groups exposed to 75 krad or more. The severity of this response in terms of percent mortality clearly appeared to be dose dependent but only from 2 to 7 days PI. By 7 days, 100% of the oysters exposed to 200 krad had died compared to 56% in the 150-krad group, 17% in the 100-krad group, and 11% in the 75-krad group.

The second time period during which numerous mortalities occurred was correlated with histopathological observations which indicated that general tissue deterioration had occurred. The second

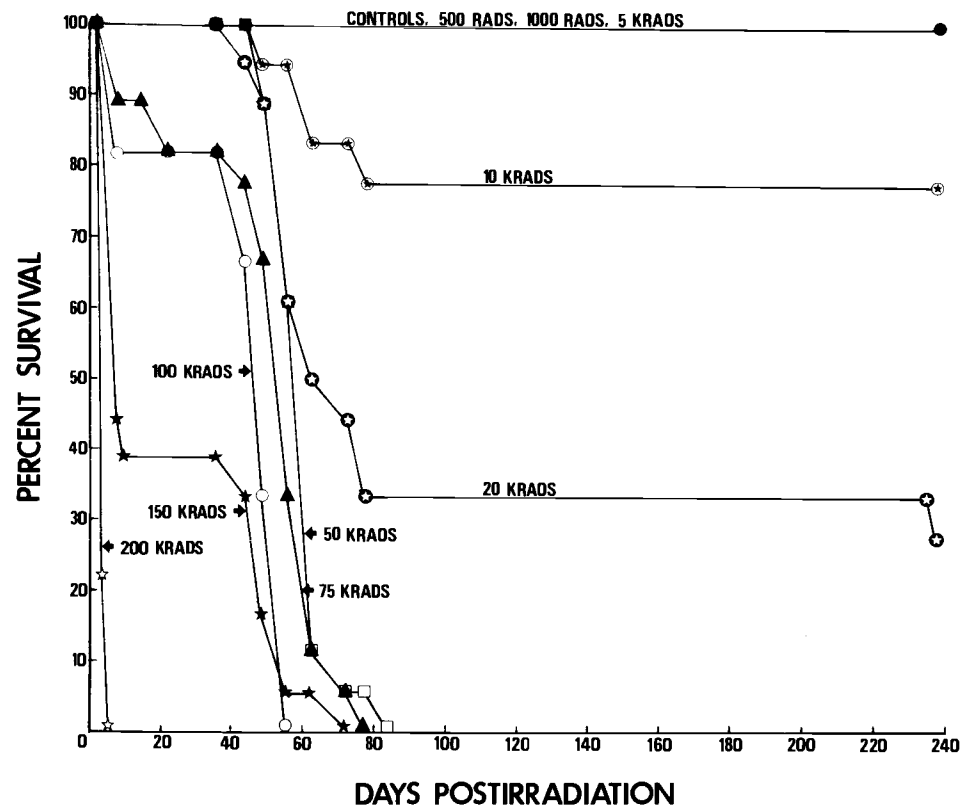


Figure 1. Postirradiation survival of oysters exposed to single total-body doses of gamma radiation ranging from 0 rads (controls) to 200 krad.



period of mortalities was termed the lethal tissue degenerative (LTD) syndrome. The LTD syndrome was manifest in the survival study from 40 to 60 days PI in groups exposed to 10 krads or more. The severity of this syndrome, in terms of percent mortality appeared to be dose independent in the groups receiving 50 krads or more. Oysters exposed to less than 50 krads exhibited a dose dependent response. The onset of the LTD syndrome occurred slightly earlier in the higher dose groups (75, 100, and 150 krads). All oysters that survived beyond 7 days in groups affected by the AL syndrome died later as a result of the LTD syndrome.

A second way to compare radiation sensitivity is to determine mean survival times PI. Figure 2 is a plot of log mean survival time versus log dose and is the type of plot that has been characteristically used to demonstrate radiation syndromes in mammalian radiation studies (Casarett, 1968). Since all oysters exposed to 10 and 20 krads did not die during the experimental period, the true mean survival times (MST) for these groups could not be determined. However, for comparative purposes MST's were calculated for these groups using an experimental termination point that presupposed all remaining oysters died at that point. This approximation is shown as a dashed line in Figures 2 and 3. The actual MST's for the 10 and 20 krad oysters are probably greater than those shown in Figures 2 and 3 and may eventually be determined from this study.

Three separate components were noted in the log plot of the mean survival curve (Figure 2) while the linear plot displayed only 2. The first component was the same in both figures. A large drop in

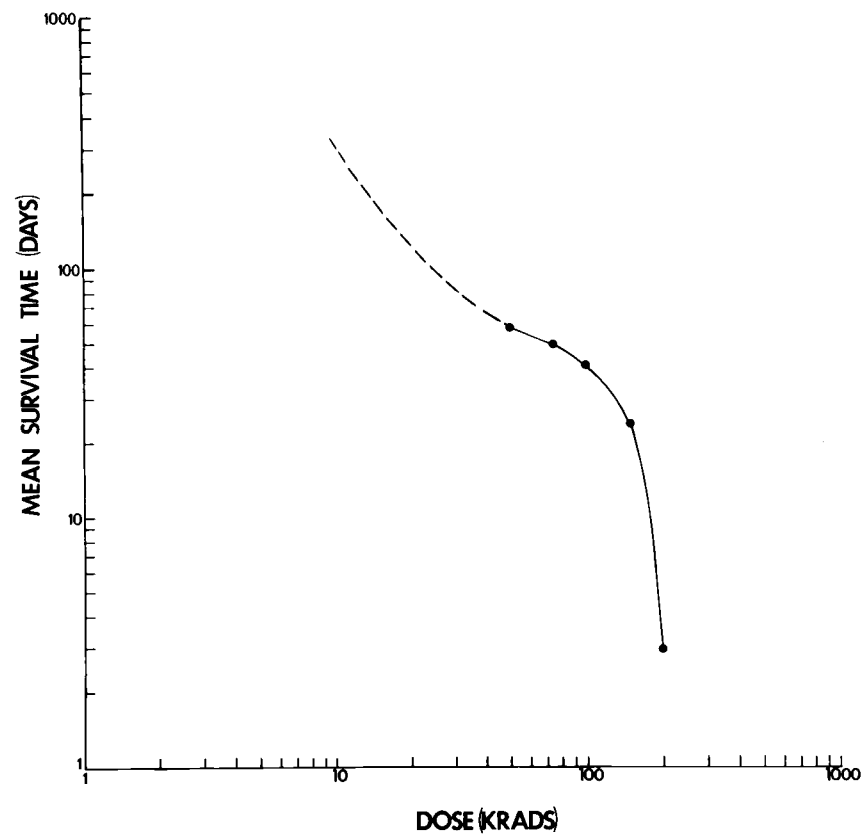


Figure 2. Relationship between dose and survival time for juvenile Pacific oysters following a single total-body exposure of gamma radiation (log-log plot). Dashed line represents estimates of mean survival times for oysters exposed to 10 and 20 krads.

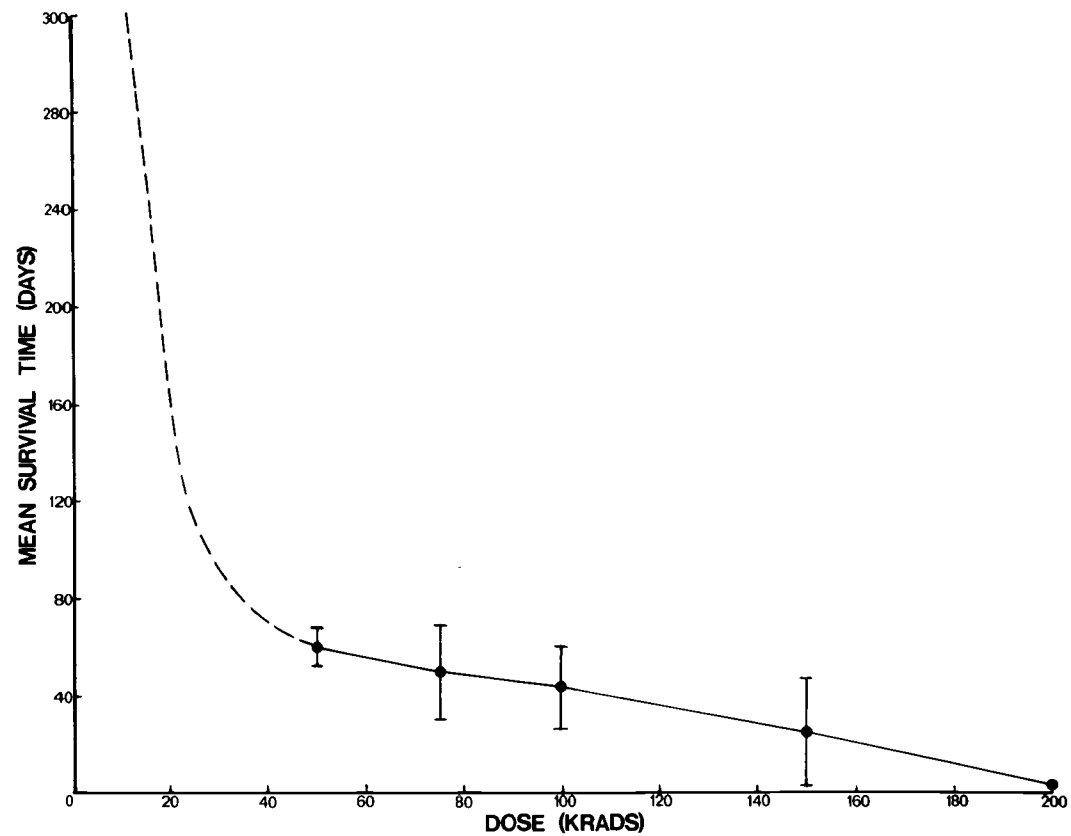


Figure 3. Relationship between dose and survival time for juvenile Pacific oysters following a single total-body exposure of gamma radiation (linear plot). Dashed line represents estimates of mean survival times for oysters exposed to 10 and 20 krads.

the MST was noted from 10 to 50 krads. The dose dependent character of this region is somewhat misleading since mortalities in both the 10 and 20-krad groups were thought to be due to the LTD syndrome. In other words, an examination of the data revealed that most mortalities observed in these groups occurred from 40 to 60 days PI or during the period attributed to the LTD syndrome.

The second component of the log mean survival curve (Figure 2) was observed from 50 to 100 krads. In this dose range, all oysters died as a result of the LTD syndrome. The third constituent observed in Figure 2 occurred in the dose range of 100 to 200 krads. Here the LTD syndrome was of decreasing importance while the AL syndrome became paramount.

Figure 3 indicates an approximately linear relationship between dose and survival time from 50 to 100 krads. An average decrease in MST of 0.29 days per krad exposure was calculated from 50 to 100 krads. From 10 to 50 krads, an average decrease in MST of 6.0 days per krad exposure was determined.

In juvenile Pacific oysters the LD-50 dose appeared to be a complex function of time beginning at the moment of irradiation until approximately 80 days PI (Figure 4). From 80 days to the termination of the experiment 238 days PI, only slight changes were seen in the LD-50 values. During the first 80 days PI, two periods were identified during which large decreases in LD-50 were observed. These corresponded to the previously described radiation syndromes. During the AL syndrome (2 to 7 days PI) the LD-50 dose was found to drop from 200 to approximately 142 krads. Very little change occurred

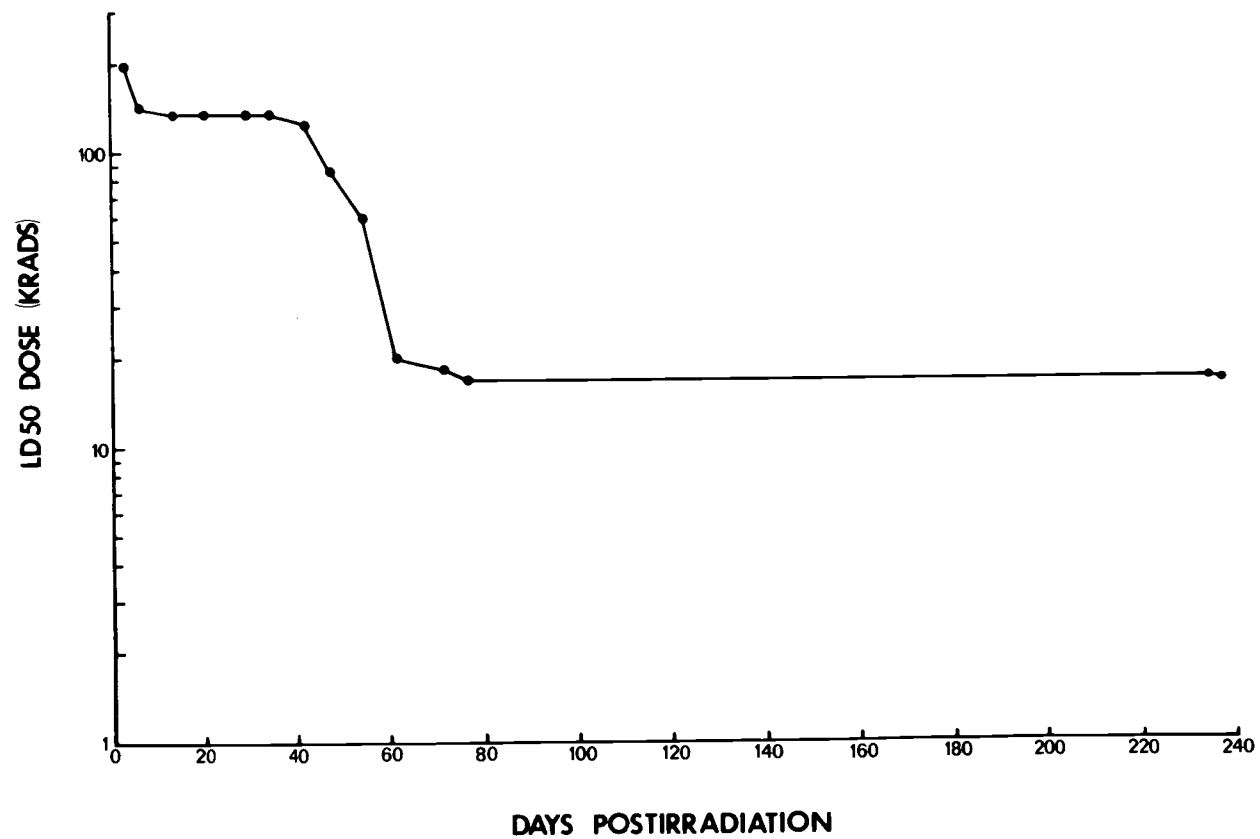


Figure 4. Relation between the LD-50 dose and time postirradiation for juvenile Pacific oysters.

in the LD-50 dose from 7 to 43 days PI. From 35 to 77 days the LD-50 dose ranged downward from 137 to 17 krads. The large drop in the LD-50 dose resulted from mortalities due to the LTD syndrome. Death of one oyster in the 20-krad group between 235 and 238 days resulted in a reduction of the LD-50 value to 16.5 krads.

#### Mean Wet Weight Postirradiation

From the time of irradiation to 30 days PI all groups in this portion of the study (control, 500 and 1000 rads, and 5, 10, 20, 50, and 75 krads) displayed a net increase in mean wet weight (MWW) (Figure 5). Between 30 and 43 days PI groups receiving doses of less than 5 krads showed little or no increase in the MWW while groups receiving doses of 10 krads or greater all showed a decrease in MWW. As stated earlier, during this time mortalities were beginning to result from the LTD syndrome. In addition, an influx of freshwater into the bay and the concomitant heavy siltation during this time may have resulted in a decreased growth rate. After 43 days PI a resumption of growth was noted in groups exposed to 10 krads or less but not in the 20, 50, or 75 krad dose groups.

The 20-krad group showed virtually no change in MWW from 43 to 72 days PI. This period corresponded to the time of maximum mortalities attributed to the LTD syndrome. After 72 days a pattern of slow steady growth was observed until termination of the experiment 238 days PI.

Oysters receiving doses of 50 to 150 krads showed a slight increase in MWW from the time of irradiation until 30 days PI. From

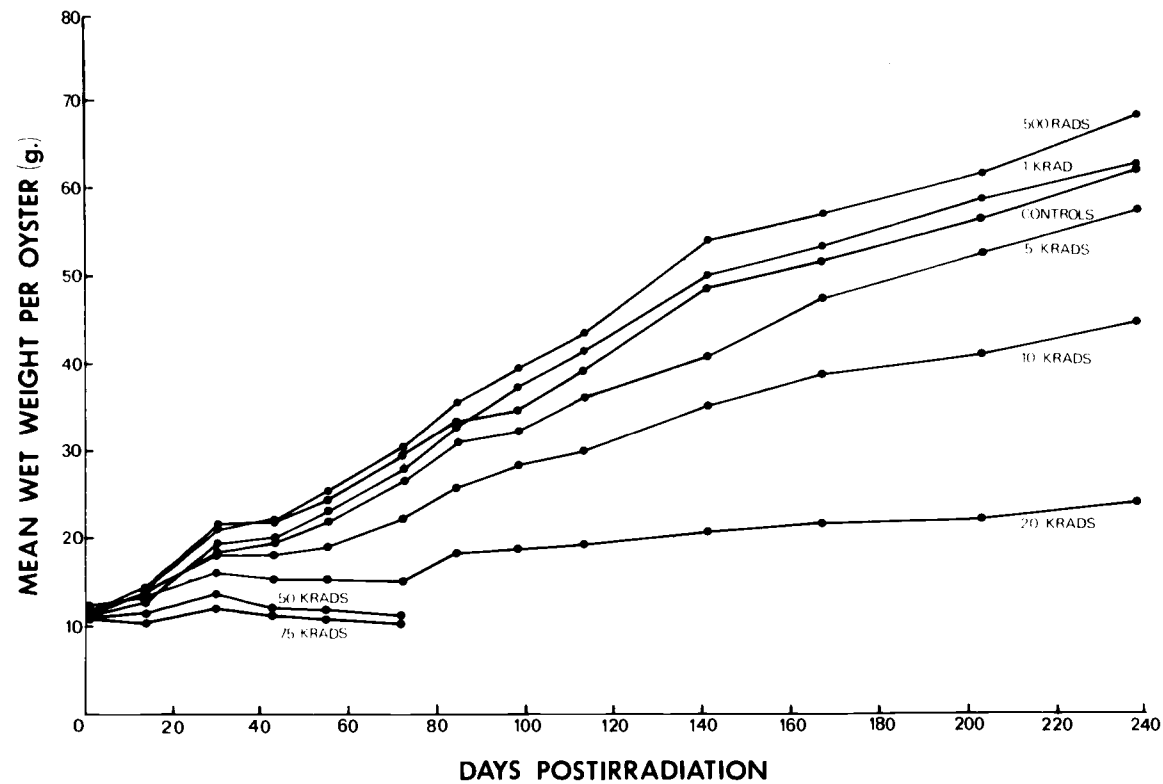


Figure 5. Mean wet weight as a function of days postirradiation for juvenile Pacific oysters exposed to single total-body gamma doses ranging from 0 rads (controls) to 75 kRads.

30 days until all animals had died, oysters in these groups exhibited a continuous decrease in MWW.

From 98 until 238 days PI the growth rate remained nearly constant in all surviving groups. Groups receiving doses of 5 krad or more appeared to exhibit a dose dependent growth pattern. Growth rate calculations for this period revealed an average increase of 0.22 g per day in the control group. During the same interval the 10 krad group increased an average of 0.14 g per day and the 20-krad group 0.05 g per day.

Prior to 167 days PI no statistical test of significant difference of MWW between groups was conducted. Beginning 167 days PI, oysters in each group were weighed individually. This allowed determination of within group variation in terms of standard error (SE). The only group that differed significantly in MWW from the control group from 167 until 238 days PI was the 20-krad group. Furthermore, the MWW of the 20-krad group differed significantly from all other irradiated groups during this period. Using past growth rates to determine future MWW in each group it was predicted that the MWW of the 10-krad group may eventually differ significantly from the MWW of the control group.

A possible enhancement in growth was noted in groups exposed to 500 and 1000 rads. Although not significantly different statistically from the control group, the MWW of the 500-rad group exceeded that of the control group from 43 to 238 days PI. The MWW of the 500-rad group ranged from 0.4 g more than the control group at 43 days to 6.0 g more at 238 days PI. The MWW of the 1000-rad group exceeded



that of the controls from 98 days PI by 1.8 g. By 238 days PI the MWW of the 1000-rad group was only 1.1 g greater than that of the controls.

#### Mean Shell Length Postirradiation

Oyster shell length measurements tended to be highly variable. Groups receiving doses of 8 krads or less did not differ significantly from the controls for the duration of the experiment (Figure 6). Through the course of the experiment the mean shell lengths (MSL) of the 16 and 40-krad dose groups were found to differ significantly from the control group as well as from each other.

The MSL of the 16-krad group did not differ significantly from the control group until 34 days PI. From 26 to 68 days PI, a 0.23 cm increase in MSL was observed in the 16-krad group while during the same period the MSL of the control oysters increased by 1.57 cm. During this period the LTD syndrome was observed histologically in this group. From 68 to 96 days PI, the MSL of the 16-krad group showed an increase of 1.70 cm. During this time an increase of 1.76 cm occurred in the controls. It should be noted however, that the MSL of the 16-krad oysters was significantly less than that of the controls throughout this period. From 96 to 321 days PI the 16-krad group showed an increase of 0.63 cm in MSL while the controls increased by 2.55 cm during the same period.

The MSL of the 40-krad group was significantly less than that of the controls from 34 to 196 days PI and significantly less than the 16-krad group from 83 to 196 days PI. From 26 to 138 days PI no

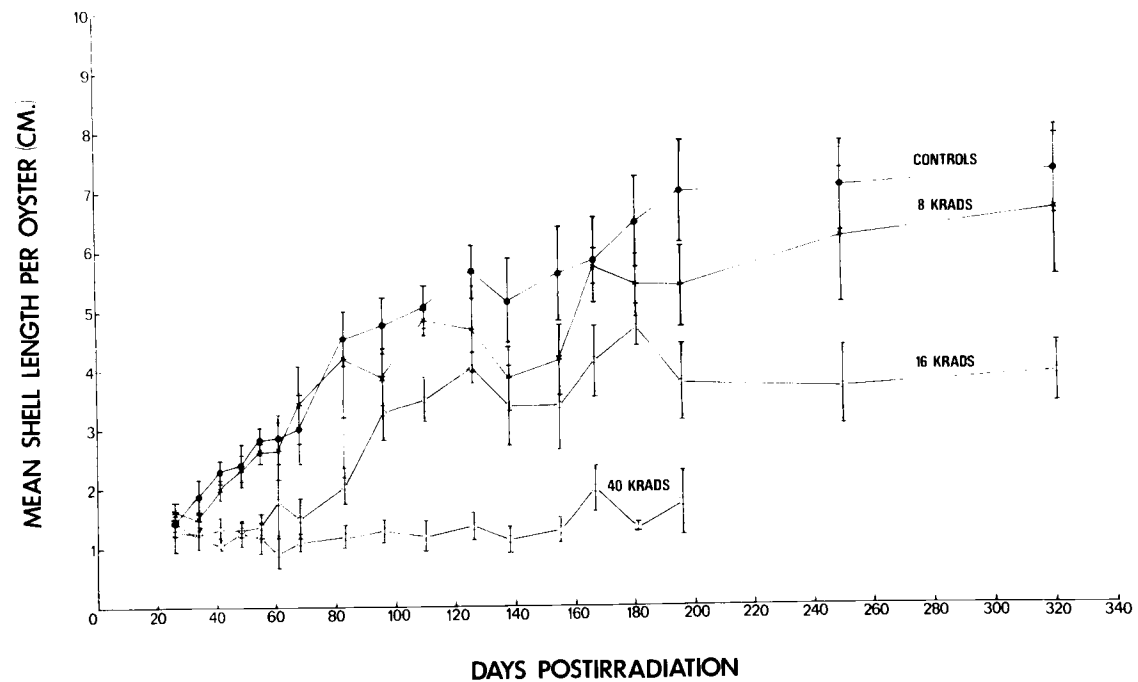


Figure 6. Relationship between mean shell length and days postirradiation for juvenile Pacific oysters exposed to single total-body gamma radiation. Doses included 0 rad controls and 8, 16, and 40-krad groups.

change in shell length was observed. A slight increase was noted between 138 and 196 days PI. Histopathological examinations of these animals revealed that they were more severely affected by the LTD syndrome than were the 16-krad group oysters (discussed below). This may explain the long period during which no shell growth was observed.

### Histopathological Studies

Histopathological analyses of the digestive tissues from 6 to 321 days PI revealed degenerative changes in the 5, 8, 16, and 40-krad groups. Tissues affected in the 16 and 40-krad groups included epithelial surfaces of the stomach, gut, collecting ducts (CD), and digestive tubules (DT) while only the DT were apparently affected in the 5 and 8-krad groups. In the 16 and 40-krad groups the most severe degeneration occurred from 30 to 60 days PI. The observed tissue degeneration was thought to be related to the LTD syndrome because it coincided in time with that syndrome described previously in the survival study.

A tissue recovery sequence was observed in the stomach, gut, CD, and DT of most oysters exposed to 16 krads and in some oysters exposed to 40 krads. Tissue regeneration was first observed in the DT and subsequently in the stomach, gut, and CD. Digestive tubule regeneration was first observed 26 days PI in 16-krad oysters and 34 days PI in 40-krad oysters. Complete recovery of the DT had occurred by 41 days PI in the 16-krad group and by 83 days PI in the 40-krad oysters. Regeneration of the stomach, gut, and CD was first observed

34 days PI in the 16-krad group and 48 days PI in the 40-krad oysters. In the 16-krad group, complete recovery of the gut and CD was observed by 48 days PI. Recovery of the stomach proceeded at a slower rate and was not completed in the 16-krad oysters until 61 days PI. In the 40-krad oysters, complete recovery of the CD occurred by 83 days PI. The stomach and gut of the 40-krad group appeared normal by 96 days PI.

The histopathological descriptions of the LTD syndrome and the subsequent recovery sequence in the stomach, gut, CD, and DT are presented below. Discussion of the LTD syndrome includes descriptions of the 5, 8, 16, and 40-krad groups from 6 to 96 days PI. The regenerative sequence is described for the 16 and 40-krad oysters.

1. Lethal Tissue Degenerative Syndrome (5, 8, 16, and 40 krads)

- 6 days

Numerous secretory and absorptive cells of the DT (hereafter called DT "functional" cells) appeared to be undergoing distal fragmentation (lysing or loss of integrity of distal surface of cell) in the 40-krad group. As a result, the DT lumina contained a slightly greater amount of eosinophilic cellular debris than the controls. No change from the controls was noted in the DT of the 5, 8, or 16-krad groups.

Epithelial tissues of the stomach, gut, and CD in the 5, 8, 16, and 40-krad oysters were indistinguishable from the control oysters at this time.

No mitotic figures were observed in the stomach, gut, CD, or DT of the four irradiated groups. In contrast, numerous mitotic figures were seen in these tissues in the unirradiated oysters.

#### 13 days

Distal fragmentation and cell sloughing of the DT functional cells was observed in both the 16 and 40-krad groups which resulted in an increased amount of eosinophilic cellular debris since the previous observation. In addition, many of the nuclei of the functional cells in the 5, 8, 16, and 40-krad groups had a crenulated appearance.

The basal epithelial cells of the stomach, gut, and CD of the 16 and 40-krad groups appeared reduced in height when compared with controls. A few gaps in the epithelial surfaces were also visible in the 40-krad group at this time. The epithelial tissues of the 5 and 8-krad oysters appeared unaffected.

Mitotic inhibition was still observed in the stomach, gut, CD, and DT of the four irradiated groups. Control oysters contained numerous mitotic figures in these tissues.

#### 20 days

By this time, the number of DT crypt cells (stem cells in the DT cell renewal system) in the 8, 16, and 40-krad groups had visibly decreased (Figures 7 and 8). Based on limited observations, no increase in necrotic crypt cells was observed and thus, it seems likely that crypt cells that survived the radiation treatment

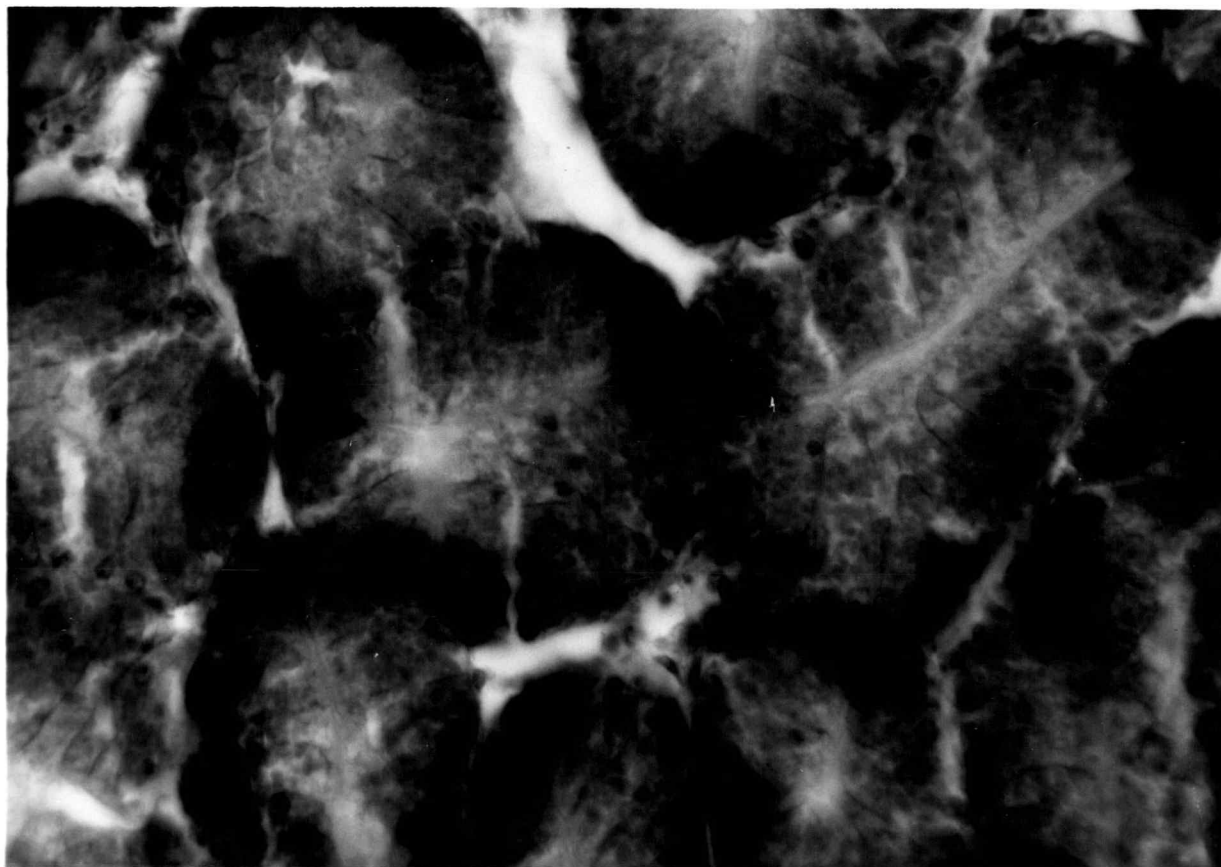


Figure 7. Normal digestive tubules. Note the dark staining "crypt regions" comprised of large, undifferentiated crypt cells. Between these regions and around the interior of the tubule, secretory and absorptive functional cells can be seen. (600 X)

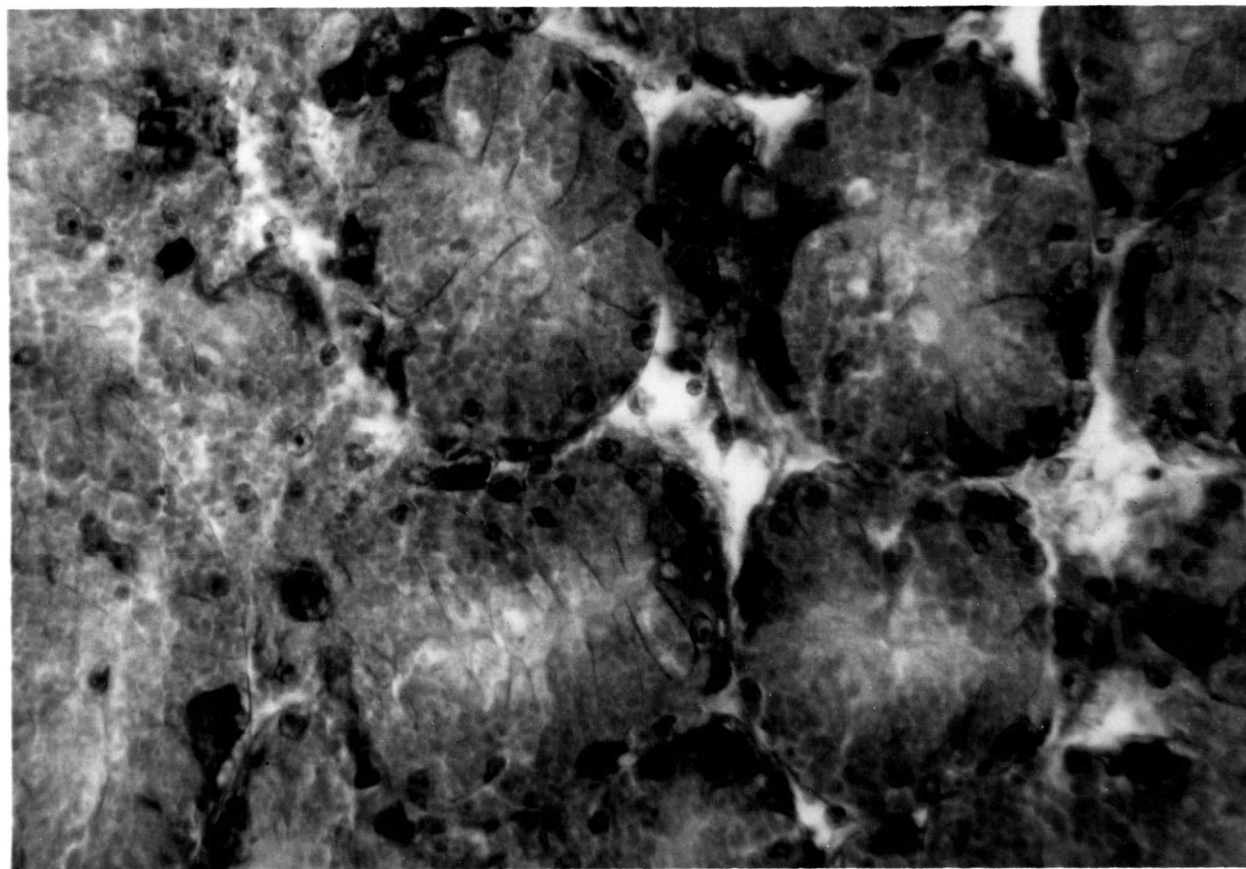


Figure 8. Degenerating digestive tubules 20 days after irradiation with 40 krads.  
Note the reduced number of crypt cells. (600 X)

eventually differentiated into DT functional cells. That phenomenon, coupled with the absence of mitotic activity in the crypt cells presumably accounted for the decrease observed in the number of DT crypt cells. The extent of the observed decrease in DT crypt cells was directly related to the radiation dose; a greater decrease in the number of DT crypt cells was seen in the 40-krad group compared to the 16 or 8-krad groups. Crenulated nuclei were present in greater numbers in all four irradiated groups; none were seen in the control oysters.

A number of small denuded regions appeared in the stomach and CD epithelial tissues of the 16-krad oysters. These tissues were more extensively denuded in the 40-krad oysters. The majority of the basal epithelial cells of the stomach, gut, and CD in the 16 and 40-krad groups had become shorter and broader (wider) than had been observed previously. In addition, the nuclei of the basal epithelial cells appeared larger than those of the controls. The epithelium of the gut, stomach, and CD of the 16 and 40-krad oysters stained more lightly than the controls from an apparent decreased affinity for stain.

No mitotic figures were observed in the stomach, gut, CD, or DT of either the 16 or 40-krad groups, but were seen in the DT of both the 5 and 8-krad groups. A larger number of pycnotic nuclei were noted in the DT of one oyster exposed to 8 krads.



26 days

Deterioration of the DT had continued in both the 16 and 40-krad groups. Nearly denuded or completely denuded DT were occasionally seen in the 40-krad group. Other DT in 40-krad oysters were still lined with a few crypt cells and in some cases, a few functional cells were still present. Some of the entirely denuded DT in the 40-krad group were completely filled with leukocytes (Figure 9). The histologic appearance of the DT in the 16-krad group was similar although less severe than that of the 40-krad group. A greater number of DT crypt and functional cells remained in the 16-krad group. Crenulated DT functional cell nuclei were not observed in the 16 and 40-krad groups because of the reduced numbers of functional cells. Most functional cells containing crenulated nuclei had been sloughed. Many of those remaining appeared anucleate.

Little change was observed in the stomach, gut, and CD from the previous observation. A decrease in the size of these organs was visible in 40-krad oysters. In addition, the CD of 40-krad oysters appeared to have become severely convoluted; perhaps as a result of uneven loss or shrinkage of cells in one area of the tissue.

No mitotic figures were observed in the stomach, gut, CD, or DT of oysters exposed to 40 krads. A few mitotic figures were now seen in the region of the DT crypt cells in the 16-krad oysters. An increased number of pycnotic nuclei was also observed in regions of mitotic activity of the 16-krad group which suggests that following mitotic inhibition, some of the irradiated cells were unable to

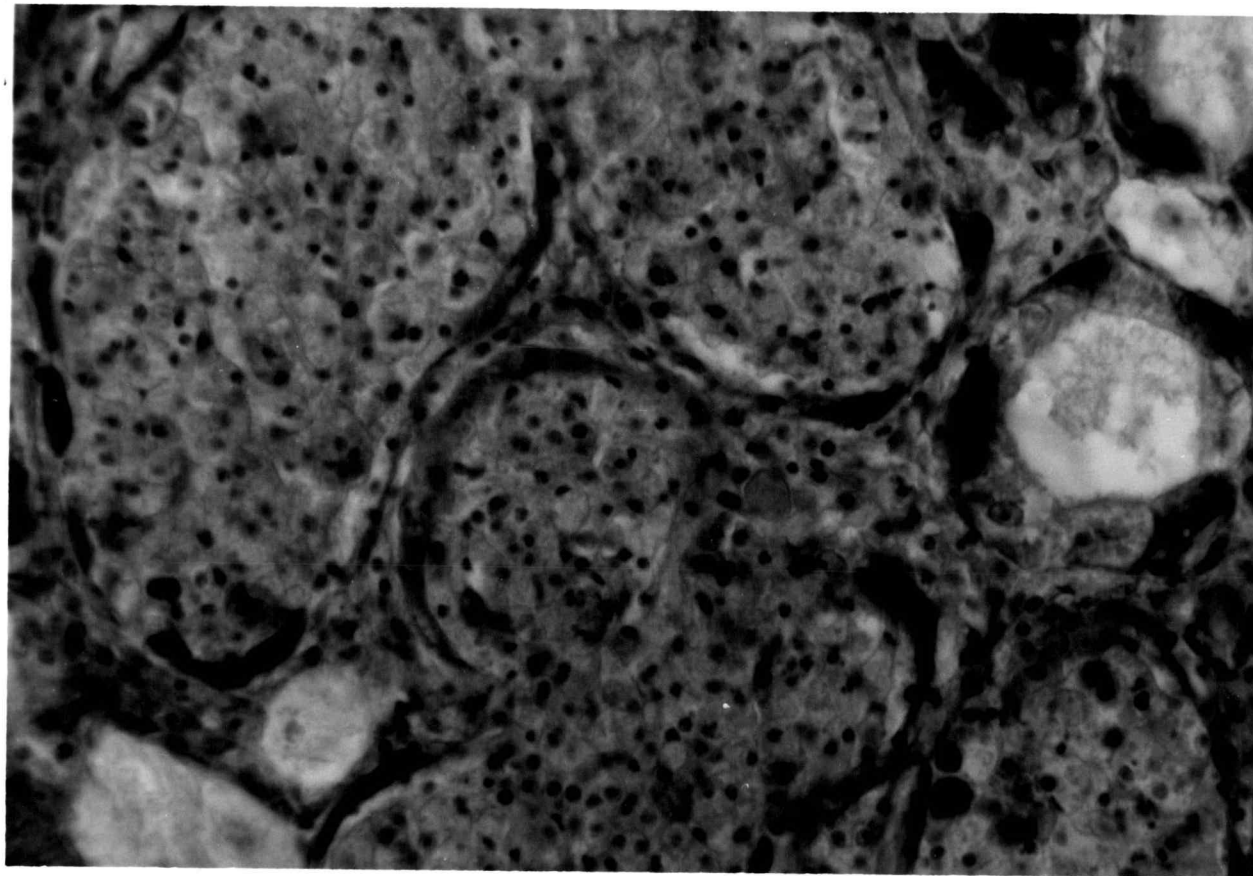


Figure 9. Further degeneration of the digestive tubules 26 days after irradiation with 40 krads. Nearly all crypt and functional cells have been lost and the lumina are filled with leukocytes and cellular debris. (600 X)

complete a successful mitotic division. These aborted attempts at mitotic division were later manifested as degenerating or pycnotic nuclei.

Large numbers of mitotic figures were observed in the stomach, gut, CD, and DT of oysters exposed to 5 and 8 krads. Subsequently, the digestive tissues of oysters in these two groups appeared normal.

#### 34 days

Little change in the DT was noted from the previous observation of the 40-krad group except that early stages of the recovery sequence were evident at this time. In the 16-krad group, the repair sequence was observed to be well advanced (the recovery sequence is reported below).

Generally, few changes had occurred in the stomach, gut, or CD epithelium in the 16 and 40-krad oysters. However, in the 40-krad group some degenerate stomach basal epithelial cells had eosinophilic bulbous protrusions projecting from the distal end of the cell (Figures 10 and 11). Mix and Sparks (1970) described similar spherical, basophilic protrusions on the distal portions of stomach epithelial cells 24 to 48 hours PI in oysters exposed to 20 krads. In the present experiment the distal protrusions were seen in some of the 40-krad oysters with severely degenerated stomach epithelium. The repair sequence had begun in the stomach, gut, and CD of the 16-krad oysters and is described below.

Mitotic figures were observed in the DT of the 40-krad oysters and in the stomach, gut, CD, and DT of the 16-krad oysters. One

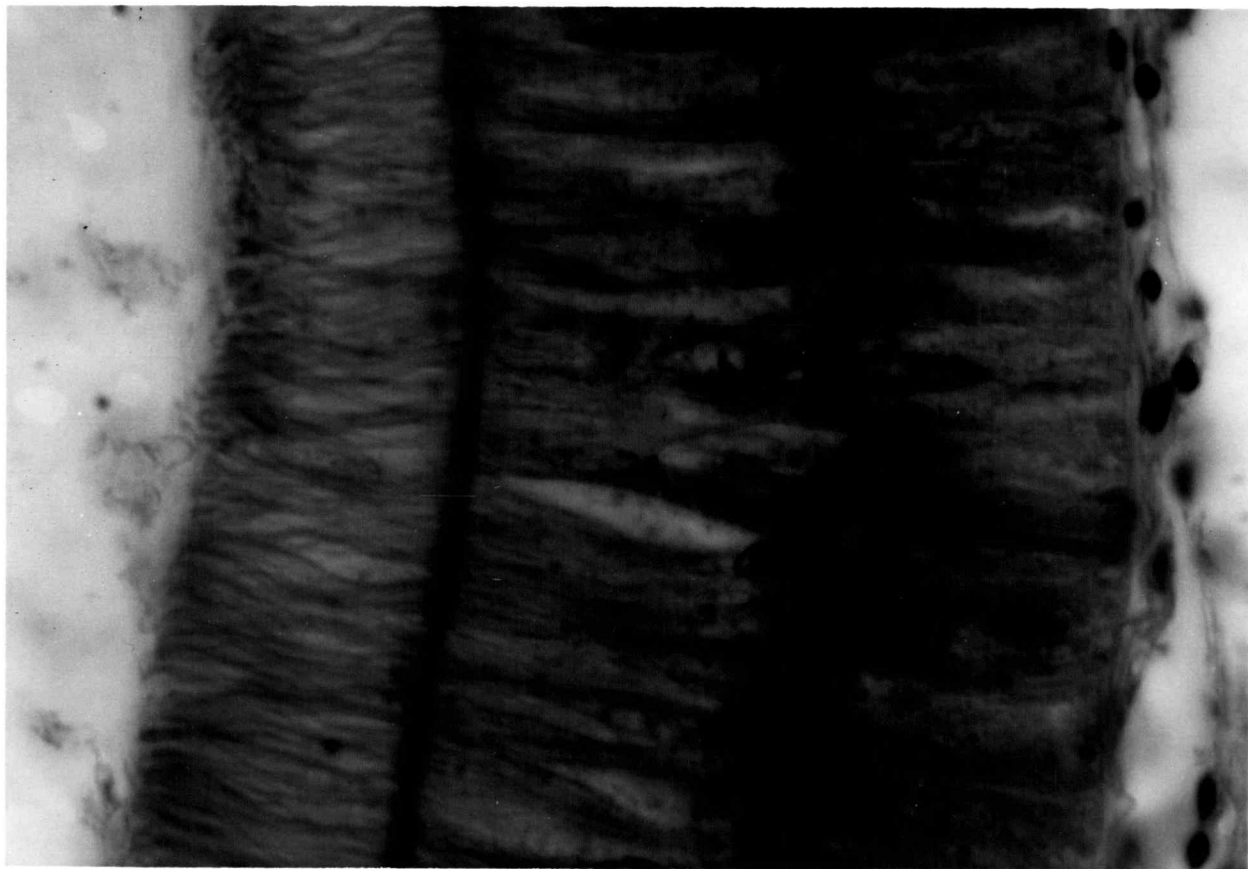


Figure 10. Normal stomach epithelium. (1500 X)

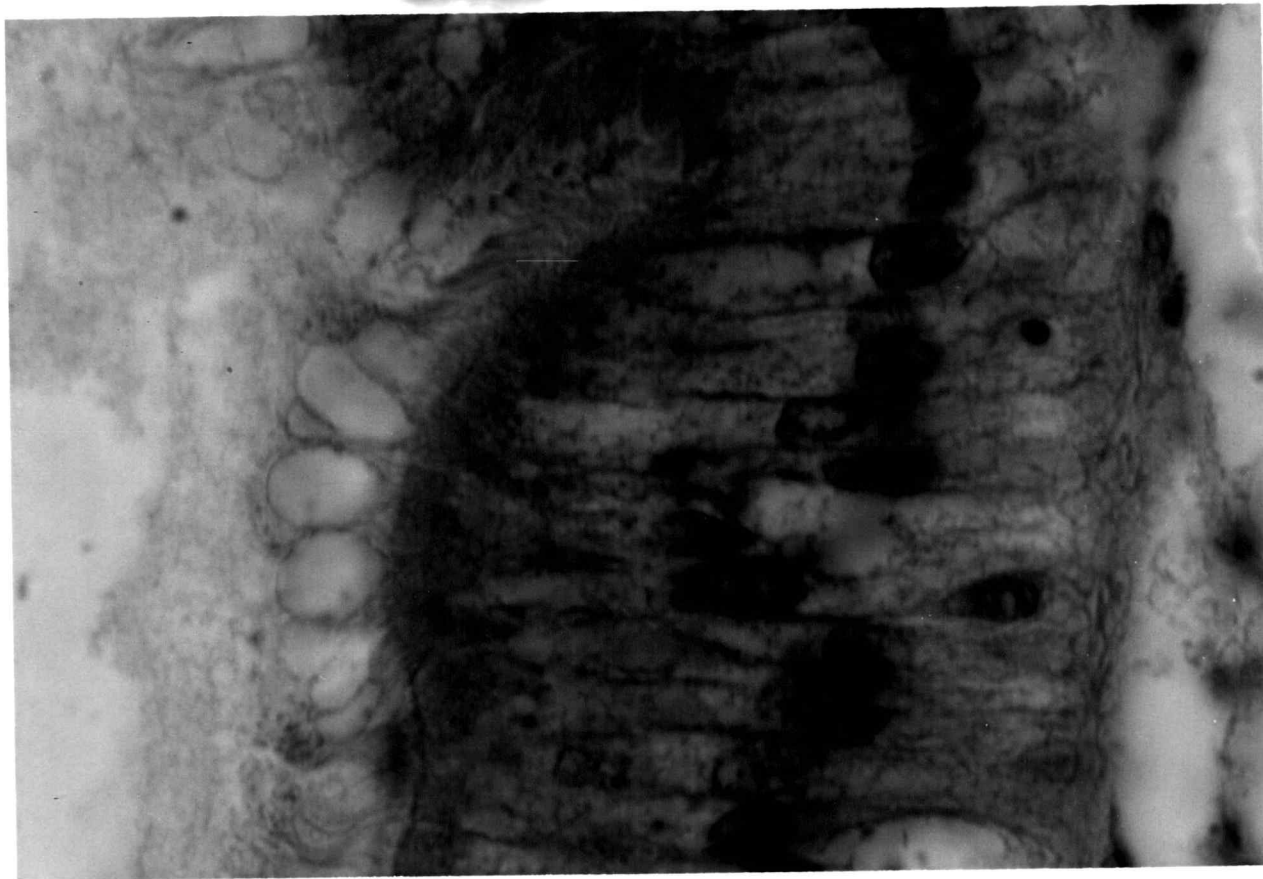


Figure 11. Bulbous protrusions from distal surface of stomach epithelial cells (34 days post irradiation, 40 krads). Note decrease in the number of nuclei. (1500 X)

oyster exposed to 16 krads had a large number of pycnotic nuclei in the epithelial surface of the DT.

#### 41 days

Tissue recovery processes were observed in two oysters while the other two oysters in the 40-krad group showed progressive degeneration associated with the LTD syndrome. The DT of the degenerating oysters had not changed since the previous observations. Numerous denuded DT were filled with cellular debris and leukocytes.

A greater degree of architectural disorganization was seen in the stomach, gut, and CD of the 40-krad oysters. Portions of the gut and CD were completely denuded in these oysters. The previously described bulbous protrusions from the distal surface of the stomach epithelial cells were seen and similar protrusions were also observed in gut epithelial cells of 40-krad oysters.

Mitotic figures were present in the DT of the recovering 40-krad oysters along with a concomitant increase in the number of pycnotic nuclei. No mitotic figures were observed in any digestive tissues in the two degenerating 40-krad oysters. Numerous mitotic figures were seen in the stomach, gut, CD, and DT of the 16-krad oysters.

#### 48-96 days

During this time period, recovery processes of the stomach, gut, CD, and DT had occurred in varying degrees in 21 of 24 oysters irradiated with 40 krads. The remaining three oysters exhibited

changes indicative of continuing degeneration of these tissues including regions of highly disorganized tissue in the stomach, gut, and CD epithelial surfaces accompanied by a considerable reduction in the size of these organs (Figures 12 and 13). Few further changes in the DT were noted. Due to the severe degeneration of the digestive tissues, accompanied by an apparent failure of the recovery mechanisms, it was assumed these three oysters would have died shortly after 96 days.

## 2. Digestive Tissue Recovery Sequences (16 and 40 krads)

### Digestive Tubules

a. 16 krads. The first indication of DT recovery in the 16-krad oysters was the presence of scattered mitotic figures in the crypt cell regions at 26 days PI. As noted earlier, the crypt cells are easily distinguished as basophilic undifferentiated cells located in groups or clumps near the basement membrane. One of the oysters examined 26 days PI had a large number of pycnotic nuclei in the crypt cell region. Between 26 and 34 days PI it was evident that effective recovery processes were operational. During this period, scattered peripheral crypt cells increased in numbers with the result that islands of these multiplying cells were visible around the DT. In some cases basophilic crypt cells completely lined the DT by 34 days PI. Since completely denuded DT were not observed in the 16-krad group, it is assumed that the new crypt cells resulted from a proliferation of surviving crypt cells. Numerous pycnotic nuclei

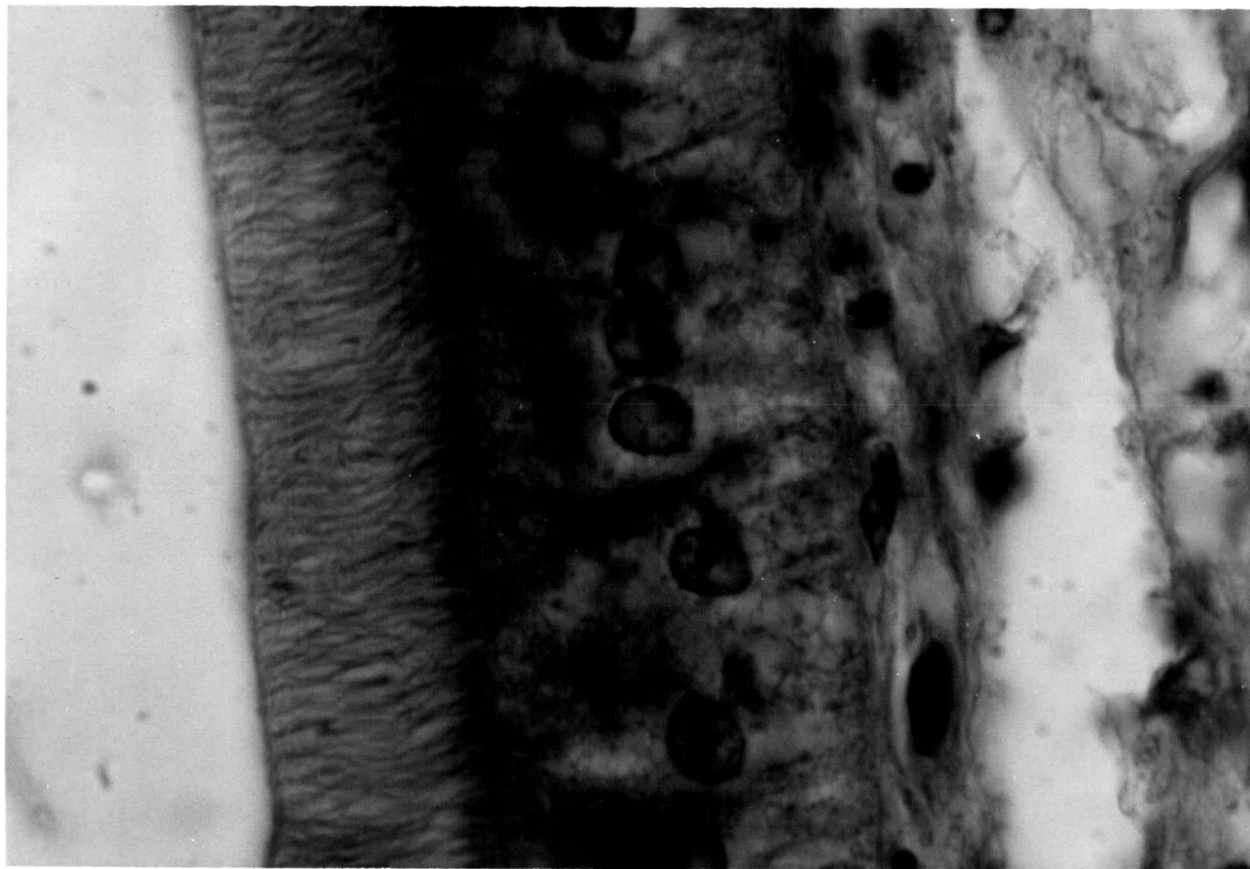


Figure 12. A portion of degenerating stomach epithelium 48 days after irradiation with 40 krads. Note a general disorganization accompanied by decreased height and increased size of nuclei in the stomach epithelial cells. (1500 X)



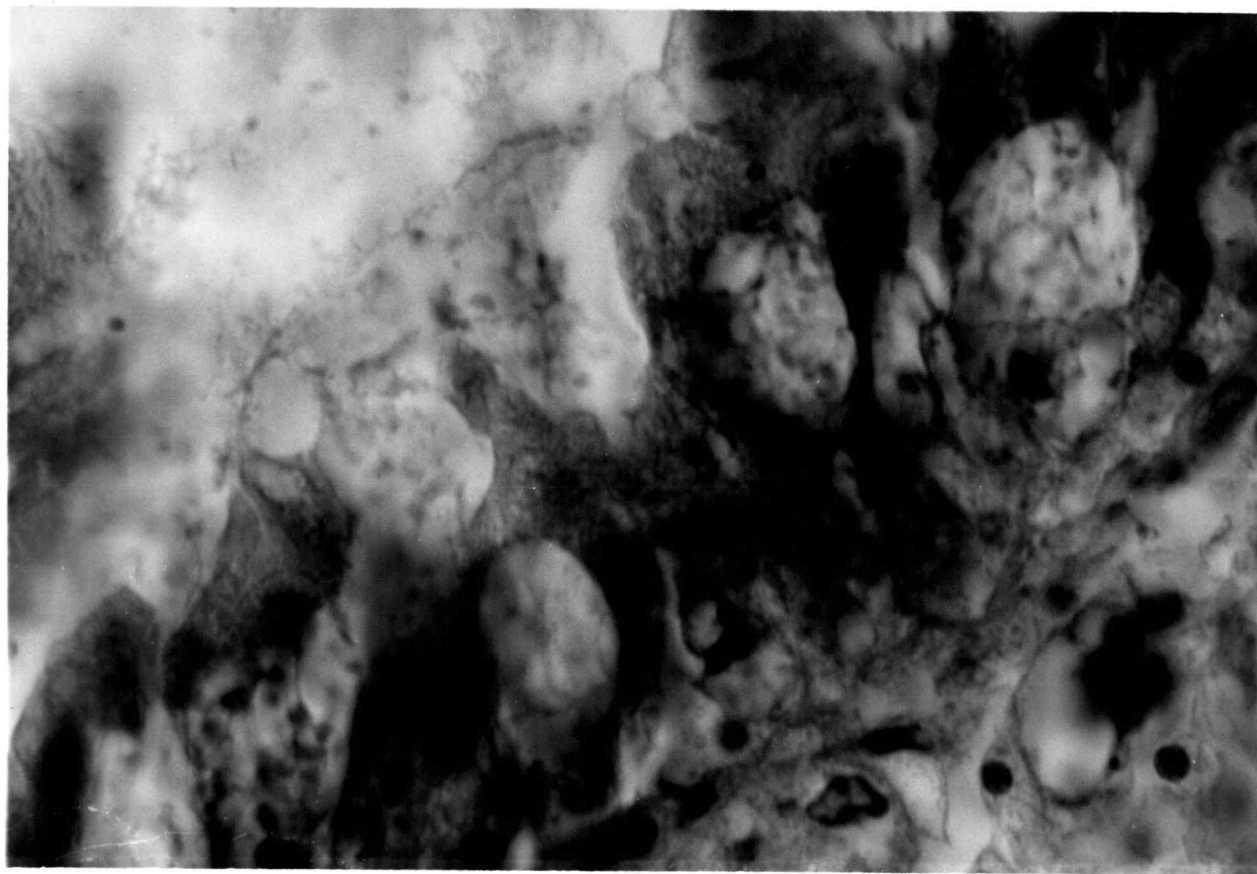


Figure 13. Complete deterioration of collecting duct epithelium (48 days post-irradiation, 40 krads). Note the acellular appearance of the epithelial tissue. (1500 X)

were observed in the crypt cell regions at 34 days PI. By 41 days PI and until the experiment was terminated after 321 days, the DT of the 16-krad oysters appeared normal.

b. 40 krads. In the 40-krad oysters, signs of recovery first were evident when a slight increase in the number of crypt cells was noted at 34 days PI. Prior to 34 days PI, completely denuded DT and DT lined with only a few remaining crypt cells were observed. In most of these oysters, all the functional cells were lost. By 41 days PI there was substantial increases in the number of crypt cells, mitotic figures, and pycnotic nuclei (Figure 14). By 48 days PI, the proliferating clumps of crypt cells appeared to be migrating around the periphery of the DT, in effect producing a new epithelial lining. Some DT by this time had been completely reepithelialized with the crypt cells. This process appeared similar to the DT repopulation sequence described by Mix and Sparks (1971) in oysters exposed to 20 krads. In their studies, the entire cell population of the repopulated DT consisted only of large, undifferentiated crypt cells. In the present experiment, by 55 days PI, differentiation of some of the crypt cells into functional cells apparently occurred since there was a significant increase in the number of functional cells. Evidence that crypt cells differentiated into functional cells comes from the fact that no mitoses were ever observed in the functional cells and crypt cells were the only other cell population in the DT. From 55 days to 83 days PI a continued increase in both the number of crypt cells and functional cells was noted. Wide variations in the

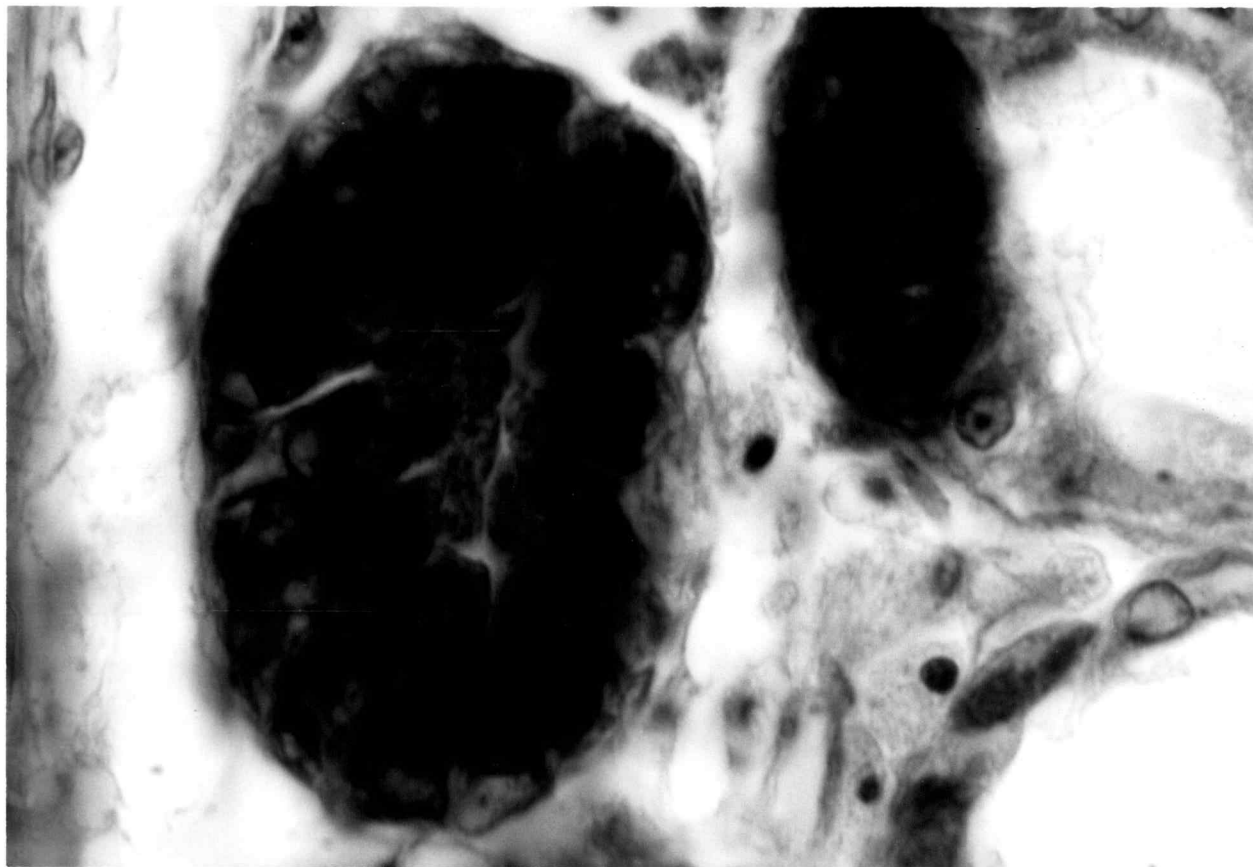


Figure 14. Repopulated digestive tubule 41 days after irradiation with 40 krads. Note the entire cell population is comprised of undifferentiated crypt cells. Also note the developing cell nest in the digestive tubule at the upper right. (1500 X)

rate of repopulation were observed between different oysters as well as between different DT in the same oyster. However, by 83 days PI, the DT of most of the 40-krad oysters appeared to have been restored to their normal appearance. No alterations of this normal appearance were observed from 83 days until all the 40-krad oysters had been sampled 196 days PI.

#### Stomach, Gut, Collecting Ducts

a. 16 krads. The recovery sequence for the stomach, gut, and CD followed a similar pattern in each of these tissues, but the rate of recovery and time of onset of the regenerative processes varied. Regenerative processes in these tissues were first noticeable by 34 days PI when scattered tightly packed clusters or islands of small basophilic cells were observed in the epithelial lining of all these tissues (Figure 15). Those new cells appeared considerably smaller than the normal epithelial cells of these tissues. The nuclei of the small basophilic cells were smaller and more oval-shaped than those of the surviving epithelial cells. In addition, a few mitotic figures were observed in the islands of basophilic cells. By 41 days PI, large numbers of mitotic figures were observed in the islands of new epithelial cells. Also, it was now clear that as the new epithelial cells matured, their width and height increased which, coupled with the continued mitotic proliferation, appeared to cause the cells to migrate around the epithelium. In the stomach, gut, and CD, the proliferating migrating regions of new cells



Figure 15. Repopulation of a collecting duct with small basophilic cells 34 days following exposure to 16 krad. Note that the basophilic cells are much smaller and have smaller nuclei than the surviving epithelial cells. Also note that differentiation of crypt cells into functional cells has occurred in the digestive tubule at the left. (1500 X)

continued to displace the pre-irradiated epithelial tissues until these organs were completely reepithelialized. Complete reepithelialization of the gut and CD was observed by 48 days PI. Reepithelialization of the stomach occurred later and was not completed until 61 days PI.

b. 40 krads. The recovery sequence followed the same pattern described for the 16-krad oysters except that a much longer period of time was required for complete reepithelialization of the tissues. That delay was assumed to be due to the greater amount of radiation damage to the tissues and/or a more prolonged inhibition of mitosis. The first signs of recovery of the stomach, gut, and CD epithelial tissues were indicated by the presence of tightly packed islands of small basophilic cells (same type as described for 16 krads). These small islands of cells appeared 48 days PI and were located within the old epithelial lining of the stomach, gut, and CD (Figure 16). Proliferation and maturation of the new epithelial cells located in these islands resulted in migration of the new cells around the old epithelium (Figure 17). The process of reepithelialization appeared completed by 83 days in the CD. Complete reepithelialization was not noted in the stomach and gut until 96 days PI. From 55 days through 83 days PI, leukocytic infiltrations into the stomach epithelium were observed. The leukocytes may have phagocytized dead and dying epithelial cells which were being replaced by the new epithelial tissues.

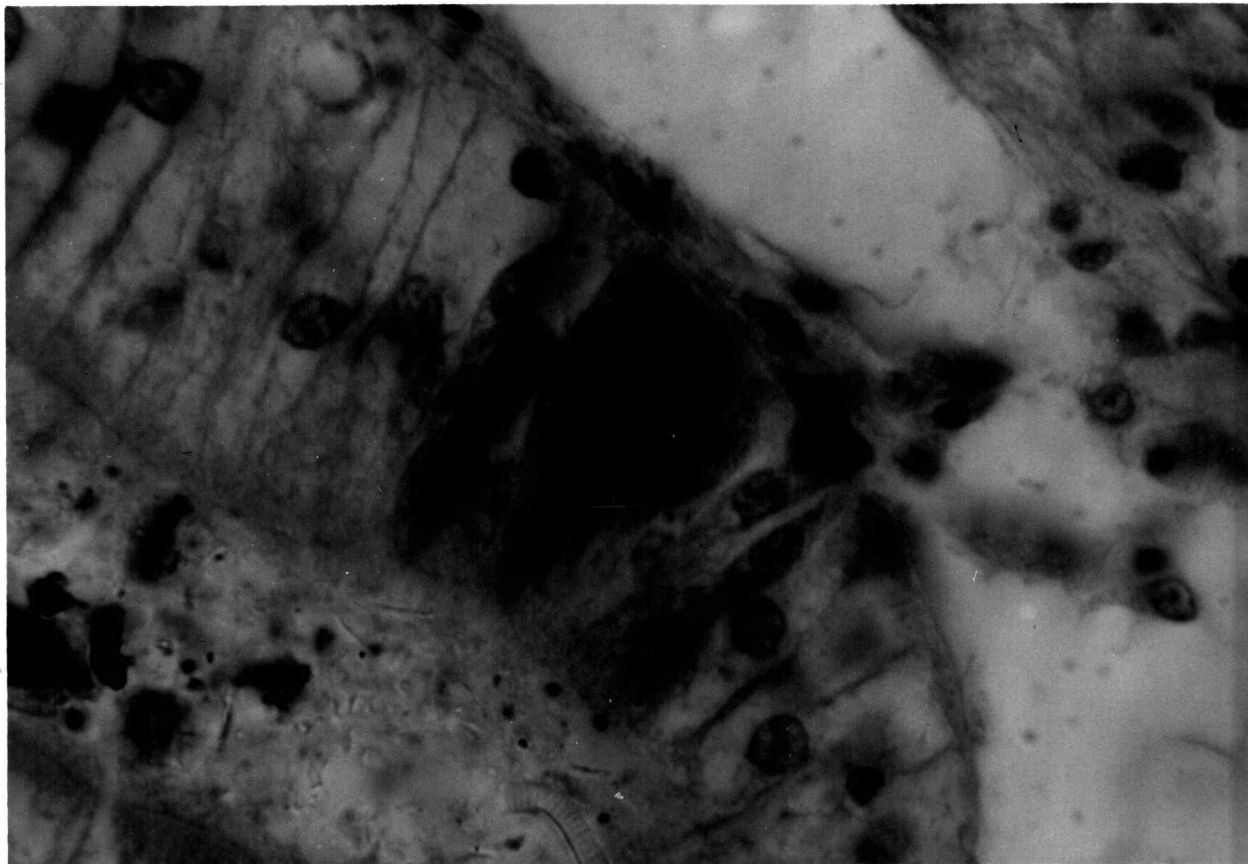


Figure 16. Early stages of cell repopulation of the gut, 48 days following exposure to 40 krads. Small basophilic cells appeared to be proliferating from a "cell nest." (1500 X)

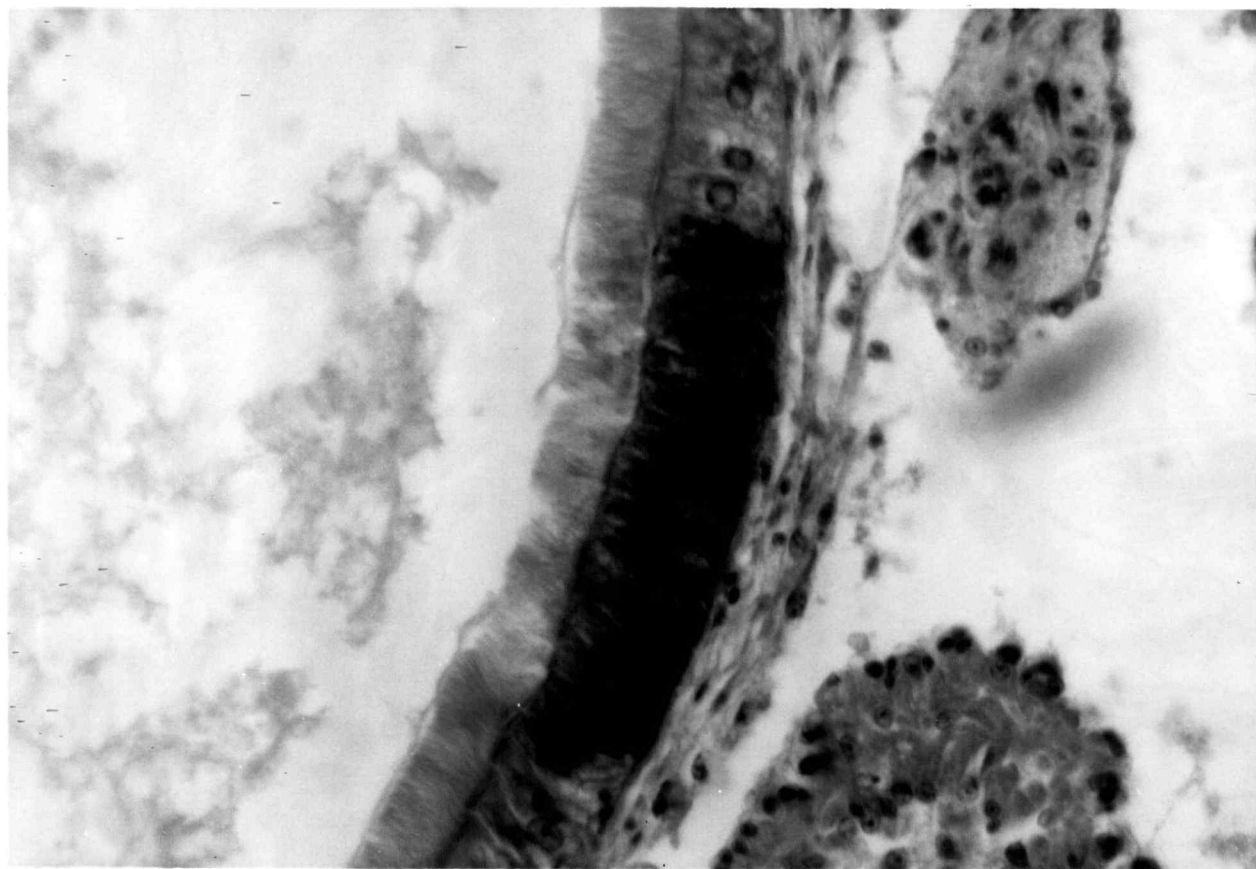


Figure 17. More advanced stage of repopulation in the stomach epithelium 48 days after irradiation with 40 krads. Note the tightly packed nature of the new epithelial cells. (600 X)



Tables I and II summarize the syndromes associated with tissue degeneration and regeneration in the digestive tubules, stomach, gut, and collecting ducts.

Table I. Digestive tissue degenerative syndrome and repair sequence-digestive tubules.

Time of Observation, Days Postirradiation	Degenerative Syndrome and Repair Sequence-DT	
	16-krad Group	40-krad Group
6 days	Mitotic inhibition of crypt cells	Mitotic inhibition of crypt cells; increased number of pycnotic nuclei; distal fragmentation of some functional cells resulting in eosinophilic debris in lumina
13 days	Distal fragmentation and cell sloughing resulting in increased eosinophilic debris in lumina; crenulated nuclei functional cells	Crenulated functional cell nuclei
20 days	Decreased number of crypt cells	Decreased number of crypt cells
26 days	Few mitotic figures and increased number of pycnotic nuclei observed in crypt cell regions	Some DT nearly or completely denuded; some leukocytic infiltration into damaged lumina
34 days	Increased number of crypt cells; some differentiation of crypt cells into functional cells; numerous pycnotic nuclei	Mitotic figures observed in crypt cell regions of some oysters
41 days	Appeared normal	Increased number of crypt cells and mitotic figures in recovering oysters. Progressive deterioration and leukocytic infiltration in non-recovering oysters

Table I (Continued)

Time of Observation, Days Postirradiation	Degenerative Syndrome and Repair Sequence-DT	
	16-krad Group	40-krad Group
48 days	Appeared normal	Reepithelialization of DT with large undifferentiated crypt cells
55 days	Appeared normal	Differentiation of crypt cells into functional cells
83 days	Appeared normal	Appeared normal

Table II. Digestive tissue degenerative syndrome and repair sequences-stomach, gut, and collecting ducts.

Time of Observation Days Post- irradiation	Degenerative Syndrome and Repair Sequence- Stomach, Gut, CD	
	16-krad Group	40-krad Group
6 days	No mitotic figures stomach, gut, or collecting ducts	Same as 16-krad group
13 days	Stomach, gut, CD epithelial cells reduced in height	Stomach, gut, CD epithelial cells reduced in height; few gaps epithelial surfaces these tissues
20 days	Numerous small denuded regions observed in stomach and CD epithelium; epithelial cells of stomach shorter and wider and had decreased affinity for basophilic stain	Same as 16-krad group
26 days	No change	Stomach, gut, and CD decreased in size; CD appeared convoluted
34 days	Scattered tightly packed islands of small basophilic cells observed in epithelial lining of stomach, gut, and CD	Eosinophilic bulbous protrusions observed projecting from the distal end of the stomach epithelial cells
41 days	Reepithelialization of stomach, gut, and CD occurring	Portions of the gut and CD completely denuded. Bulbous protrusions observed in degenerating portions of gut
48 days	Complete reepithelialization of the gut and CD had occurred	Scattered tightly packed islands of small basophilic cells observed proliferating in epithelial lining of stomach, gut, and CD in recovering oysters; progressive deterioration of stomach, gut, and CD of non-recovering oysters

Table II (Continued)

Time of Observation Days Post- irradiation	Degenerative Syndrome and Repair Sequence- Stomach, Gut, CD	
	16-krad Group	40-krad Group
61 days	Complete reepithelializa- tion of the stomach had occurred	No change
83 days	Stomach, gut, and CD appeared normal	Reepithelialization appeared completed in CD
96 days	Stomach, gut, and CD appeared normal	Complete reepithelializa- tion in stomach and gut- these tissues essentially back to normal

## DISCUSSION

Radiosensitivity and Digestive Tissue Degenerative Syndromes

The results of this study indicate that juvenile Pacific oysters are quite resistant to ionizing radiation. Remarkably efficient repair mechanisms may be partially responsible since following tissue degeneration, cellular tissue reparative processes apparently restored completely all of the digestive tissues in oysters exposed to high doses of gamma radiation.

The LD-50 dose has historically been used to measure the relative radiosensitivity of an organism. Many mammalian studies have shown that acute radiation syndromes associated with subsequent mortality normally occur within the first month after radiation exposure. Such results led to the use of a customary 30-day LD-50 dose for evaluating radiosensitivity.

The situation is different for invertebrates since maximum radiosensitivity generally occurs long after 30 days PI. Several studies have shown that the LD-50 dose decreases markedly after 30 days PI. Rice and Baptist (1971), for example, reported that in studies conducted by Price (1965), the American oyster, C. virginica, had a 30-day LD-50 dose of 100 kR and a 60-day LD-50 dose of 10 kR. Similarly, Engel (1967, 1973) reported increases in radiosensitivity after 30 days PI in five species of decapod crustaceans. In my study, the LD-50 dose for juvenile Pacific oysters decreased from the time of exposure to approximately 80 days PI. From 80 days to the

termination of the experiment at 238 days, only a slight change was noted in the LD-50 dose. The results indicate that these oysters reached a maximum radiosensitivity of approximately 17 krads by about 80 days PI; after this time little change was observed in radiosensitivity.

It seems evident that tissues and cells of Pacific oysters are considerably more radioresistant than those of mammals. Studies of other invertebrates suggest that, as a group, they are very radioresistant. For instance, in the current study, no histopathological changes were observed in oysters exposed to less than 5000 rads while in mammals LD-50 doses are commonly in the range of 300 to 800 rads. A number of theories have been proposed to explain differences in radiosensitivity of different organisms. Sparrow et al. (1963) suggested that the average interphase chromosome volume (ICV) was proportional to radiosensitivity. This was demonstrated in higher plants and lower vertebrates by Sparrow et al. (1967); as the size or volume of the chromosome increased, radiosensitivity also increased. In my experiment, no effort was made to determine the ICV of C. gigas. A second theory of radiation sensitivity states that metabolic activity of an organism is directly proportional to its radiosensitivity (Patt and Quastler, 1963). Engel (1973) showed that there was a slight correlation between respiration rate (oxygen consumption) and radiosensitivity of the shrimp, Palaemonetes pugio, and crabs, Callinectes sapidus and Uca pugilator.

Another theory of radiosensitivity (Okada, 1970) proposes that the oxygen tension in tissues affects radiosensitivity. Thus high oxygen concentration in the tissues will result in a greater number of free peroxide radicals generated during the irradiation and an increase in tissue damage. In most marine invertebrates, oxygen tension is very low compared with mammals and many bivalve mollusks possess the capability of becoming entirely anaerobic (deZwaan and Wijsman, 1976). As a result, tissue oxygen tension may be reduced to extremely low levels in oysters and this may be related to their ability to tolerate high radiation doses. It has also been suggested (Casarett, 1968) that the presence or absence of radiosensitive enzyme systems may influence radiosensitivity.

Mix and Sparks (1970) noted additional factors that may influence radiosensitivity of oysters including sex of the organism, stage in the life cycle, type of radiation, and exposure conditions. Since marine invertebrates are poikilothermic and euryhaline, temperature and salinity must also be considered. The effects of salinity and temperature on radiosensitivity of the small fish, Fundulus heteroclitus, were studied by White et al. (1967). They reported that, in general, the resistance of this fish to radiation was greater in lower salinity water; however, their resistance decreased as the temperature increased.

The tissue degenerative syndromes observed in this study were less prolonged than similar syndromes described in adult Pacific oysters by Mix and Sparks (1970) and Mix (1972). It seems likely



that the higher water temperatures of Yaquina Bay ( $10^{\circ}$ - $20^{\circ}$ C) during this study, compared to those of Puget Sound ( $9^{\circ}$ - $12^{\circ}$ C) during Mix's study may have been partially responsible for the observed differences. The higher water temperature may have resulted in an increased metabolic rate and a decreased cell turnover time which in turn initiated the earlier onset of radiation degenerative changes and more rapid recovery processes. The age of the oysters may have also contributed to the short degenerative phase and the early, rapid onset of recovery seen in this study. Mix and Sparks (1970) used three-year-old oysters in their study while in the present study seven-month-old oysters were used. The young oysters were at a stage of rapid growth when irradiated. The fact that they contained active, proliferating cell populations may have contributed to the early onset of recovery. In mammals it is well known that during certain periods of their life they are more radiosensitive than at other times. There is no reason to believe that this may not also occur in invertebrates. Finally, nutritional factors may account for some of the differences. Oysters used in the present study had access to abundant food since they were maintained in an area of Yaquina Bay known to be extremely productive for oyster growth. Mix's oysters were held in large flats receiving UV-sterilized seawater; it seems likely that UV treatment, coupled with low water temperature, would have resulted in a nutritional deficiency.

Radiation syndromes are not well defined for invertebrates. Except for the work of Mix and Sparks (1970), Mix (1972, 1976), and the present study, little work has been done relating causes of death

with pathological syndromes in higher invertebrates. Mix (1972) described histopathologically two radiation degenerative syndromes in adult C. gigas: an acute lethal syndrome observed in oysters exposed to 200-400 krads and a chronic syndrome in oysters exposed to 1-100 krads. He described the acute syndrome as consisting of many cellular and tissue alterations prior to cell fragmentation and subsequent cell death 96-144 hours PI. The chronic degenerative syndrome was described as consisting of an acute tissue response 1-9 days PI and chronic tissue degeneration that occurred from 10-59 days PI in the first experiment and from 10-180 days PI in the second experiment. Chronic degeneration was thought to be associated with long-lasting mitotic inhibition (Mix, 1972, 1976).

In the present experiment similar postirradiation degenerative responses were associated with histopathological changes and affected the survival data. There were two periods of high mortality; the first occurred from 2-7 days PI in groups receiving 75-200 krads. That was termed the acute lethal (AL) syndrome and was thought to be caused by cellular and tissue alterations (sometimes culminating in cell death), similar to those described by Mix (1972). Doses of greater than 100,000 rads are thought to cause inactivation of many substances which are needed for the basic metabolic processes of the cells and tissues (Casarett, 1968). Oysters dying within several days PI at doses of 100,000 rads or greater presumably died as a result of "molecular death." The second period of high mortality occurred after oysters received doses of 10-150 krads and was observed generally from 40 to 60 days PI. Such mortality corresponded with

severely degenerated digestive tissues observed at that time and was termed the lethal tissue degenerative (LTD) syndrome.

Mortalities resulting from the LTD syndrome may be caused by failure of normal cell renewal systems of digestive tissues. For example, mitotic inhibition of the digestive tubule (DT) cell renewal system may have resulted in denudation of portions of the DT following differentiation of all stem cells. The actual cause of death may have been due to infection or starvation stemming from the partial or complete loss of functional cells in the DT. Post-irradiation mitotic inhibition was observed in all the digestive tissues affected by the LTD syndrome; however, in the DT, differentiation of crypt cells into functional cells apparently continued since there was a decrease in the number of DT crypt cells which did not appear to be due to cell death and since no necrotic or sloughed crypt cells were seen. Thus, it appeared that they differentiated into functional DT cells. Differentiation requires only the translation of pre-existing mRNA into the cytoplasm (Steele and Lang, 1976). Since DNA is much more radiosensitive than RNA, proteins or amino acids (Okada, 1970), it is evident that DNA transcription is much more radiosensitive than the process of translation from pre-existing mRNA.

Bimodal radiation response curves have been described in survival studies of other invertebrates. Hargis et al. (1957) observed two periods of high mortality for the oyster drill, Urosalpinx cinerea, which roughly corresponded with the temporal response for the Pacific oyster in the present study. They observed

25-40% mortality from 10-14 days PI in snails receiving doses of 33-48 kR. This may correspond with the AL syndrome which occurred from 2-7 days PI in oysters receiving 75-200 krads. Their data indicated there was a second period of mortality which corresponded quite closely in time with the LTD syndrome reported for C. gigas in the present experiment. The snail mortalities ranged from 50% at 21 kR to 100% at 48 kR.

Price (1965) conducted a postirradiation survival study of C. virginica. His data showed that from 25-55 days PI mortalities ranged from 72% in oysters exposed to 5833 R to 92% in oysters exposed to 93,328 R. This again corresponds in time with the LTD syndrome reported for C. gigas in the present experiment. A clearly definable AL syndrome was not described in Price's paper.

It seems evident that postirradiation changes in body weight can be used as a measure of acute and chronic radiosensitivity in invertebrates. In most organisms PI body weight is an inverse function of the radiation dose. As the dose is increased, body weight decreases with time. In some invertebrates the situation is less clear. Low doses of radiation have been reported to stimulate growth while higher doses retarded growth. White et al. (1967) showed that larval brine shrimp, Artemia salina, exposed to 500 rads not only developed into larger adults but were more uniform in size than control brine shrimp. Larval brine shrimp exposed to 2500 rads developed into smaller adults than the controls.

In a continuous gamma exposure experiment, Engel (1967) demonstrated that crabs exposed to chronic irradiation (3.2 rads per

hour) grew at a faster rate than unirradiated crabs. Only in crabs exposed to the highest continuous dose (29 rads per hour) did he observe a reduction in the growth rate. In a similar study Baptist et al. (1976) subjected juvenile clams, Mercenaria mercenaria, and scallops, Argopectin irradians, to continuous gamma radiation. They found that the clams exposed to the lowest radiation levels (0.007-0.008 rads per hour) increased in weight faster than the controls for the first month of the experiment. They noted a decreased growth rate only in clams exposed to the highest dose levels (16-37 rads per hour). Due to experimental difficulties, growth of the clams was observed only during the first month of the 14-month study and thus their results are questionable. They also noted that radiation appeared to have no effect on the growth of scallops during the 12 weeks they were observed.

In my studies, although not statistically significant, oysters exposed to the lowest doses of radiation (500 rads, 1000 rads) appeared to show an increased growth rate. A significant decrease in growth was observed only in the 20-krad group.

#### Repair of Digestive Tissues Postirradiation

Most 16-krad oysters showed complete regeneration of injured digestive tissues following irradiation, while in the 40-krad group, tissues in only a small number of oysters recovered. The difference can be explained by considering the general effects of ionizing radiation on cell renewal systems, described by Patt and Quastler (1963). They stated that cell production may be impaired with little

change in the rate of cell decay that would lead to cell depletion; the degree that production is impaired determines the degree of cell depletion. In other words, radiation inhibits new cell production, and the size of the radiation dose determines the extent and length of mitotic inhibition. If the organism is able to survive until cell proliferation can be resumed, more or less complete restoration of the system is possible.

In this study, the DT crypt cells of the 40-krad oysters were apparently more severely impaired than those of the 16-krad group. This led to greater crypt cell depletion in the 40-krad group and as a result, fewer animals were able to survive until crypt cell proliferation could be successfully resumed. Mix and Sparks (1971) described repopulation of DT with large, undifferentiated crypt cells in oysters exposed to 20 krads. They noted that regeneration began by 50-60 days PI and continued through 90 days PI when the experiment was terminated. They did not observe differentiation of the crypt cells into functional cells. As mentioned previously, differences between their results and those of the present study were probably due to differences in water temperatures, the age of the experimental animals, and/or nutritional factors. The cooler water temperatures in which Mix and Sparks (1971) carried out their study might be expected to increase cell turnover time and in effect delay repopulation of the DT. Moreover, it is possible that differentiation of crypt cells into functional cells does not occur, or occurs more slowly at low water temperatures. Evidence supporting this conclusion comes from the fact that Mix and Sparks (1971) described

proliferation and repopulation of DT with undifferentiated crypt cells in oysters exposed to 20 krads, but never observed differentiation of these cells. In the present study differentiation was seen by 34 days PI in 16-krad oysters and by 55 days PI in 40-krad oysters.

Tissue recovery processes were also manifested in the epithelium of the stomach, gut, and CD of the 16- and 40-krad oysters. Unlike the DT, no stem cell population has been described in these epithelial tissues. Mix (1971) noted that in normal Pacific oysters, gut epithelial cell renewal involved migration of the cell nucleus to the distal portion of the cell where division subsequently occurred. In the present study, reepithelialization of these tissues in the 16- and 40-krad oysters involved proliferation of apparently undifferentiated epithelial cells. Those cells appeared to act as stem and proliferative cells in the repopulation of the stomach, gut, and CD epithelial tissues. These undifferentiated cells had a high affinity to basophilic stain and generally were smaller and had smaller nuclei than the epithelial cells of the stomach, gut, and CD of unirradiated oysters. The origin of these progenitor cells is unknown but several hypothetical sources are possible: (1) there is in the stomach, gut, and CD a previously undescribed epithelial stem cell population; (2) dedifferentiation of surviving functional epithelial cells may have given rise to the small undifferentiated cells; (3) a pluripotential cell may exist in oysters.

Although information on cell renewal of the epithelial tissues of the stomach, gut, and CD is limited, there is currently no

information that does or does not support the existence of an identifiable stem cell population in these tissues. It is possible that such cells have not yet been identified or that the normal cell renewal processes of these tissues involves dedifferentiation of functional cells. Perhaps functional cells possess the capability to dedifferentiate and then divide into small undifferentiated daughter cells which then mature to form functional epithelial cells. In the present study, during the early stages of PI recovery in oysters that had severely damaged stomach, gut, and CD epithelial tissues, repopulation was observed to occur in regions where surviving epithelial cells remained intact and not in the denuded regions of these tissues. Since no other cell type was observed in the intact areas, perhaps dedifferentiation of some functional cells did occur and these cells may have acted as stem cells. In unirradiated oysters, the epithelial cells of these tissues are so tightly packed that undifferentiated epithelial daughter cells would have been difficult to identify following mitosis.

The existence of a pleuripotential cell in oysters has also been suggested (DesVoigne and Sparks, 1969; Cheney, 1971) and "fibroblasts" have most often been mentioned with regard to pleuripotential properties. If a pleuripotential cell was associated with reepithelialization of digestive tissues, it has to be assumed that not only did these cells survive irradiation, but they also must have retained the ability to subsequently differentiate into the appropriate cell type. The possibility that pleuripotential cells were the source of the small undifferentiated cells cannot be



discounted since during tissue degeneration, the surviving stomach, gut, and CD epithelial cells in the 16- and 40-krad groups had become enlarged and had very little basophilic staining character. This appearance is not characteristic of primitive cells that are going to divide mitotically. Perhaps fibroblasts or other potential pleuri-potential cells differentiated into the small undifferentiated epithelial cells that ultimately repopulated these tissues.

Before regenerative processes in oysters can be fully understood, some basic questions concerning the regulation of growth require answers. (1) What mechanism regulates proliferation and differentiation of cells in the digestive tissues? (2) How is the size of each of the digestive tissues regulated? Following severe damage, the size and shape of each of the digestive tissues was restored to normal; this implies that some mechanism exists in the oyster which regulates the proper size and shape of each tissue relative to the size of the animal. (3) Does proliferation and differentiation of stem cells in the digestive tissues occur by symmetric or asymmetric mitosis?

Several theories have been developed concerning normal regenerative and growth regulation. Bullough (1962) suggested that the mitotic rate is controlled by a series of tissue specific mitotic inhibitors. He suggested that although external factors (hormones) may modulate the mitotic rate, the ultimate control is within the tissues themselves. Thus, the cells within the tissues produce a tissue specific inhibitor that controls the mitotic rate within the tissue. Evidence supporting this theory comes from the isolation of

mitotic inhibitors called chalones. These inhibitory substances have not been studied or identified in oysters. If they existed in the irradiated oysters, a large drop in the amount of inhibitory substance would have been expected as the number of cells in the radiation damaged tissue was decreased. This would have presumably resulted in a resumption of mitosis as the tissue attempted to restore itself. However, in radiation damaged tissues, other factors may have resulted in mitotic inhibition even after the inhibitory substance was removed.

Another major theory of growth regulation proposes that inhibition of symmetrical mitosis in an organized tissue depends on contact relations between contiguous cells (Burch and Burwell, 1965). Westermarck (1971) demonstrated that contact inhibition of mitosis functions among cultures of normal, non-neoplastic, human glia-like cells. Caster (1971) produced similar results for mouse 3T3 cells. He noted that cell to cell contact inhibits division in culture by limiting the free area and movements of the cell surface. In terms of the present study, the decreased number of epithelial cells in the digestive tissues of the 16- and 40-krad oysters PI resulted in a substantial decrease in cell to cell contact that may have led to a decrease in contact inhibition. According to this theory, this decrease in contact inhibition would have effected increased mitotic activity. However, other factors may have prevented an immediate resumption of proliferative activities.

During the early recovery stages of the DT, symmetric mitoses were clearly observed. In symmetrical mitosis, a basal cell gives

rise to two identical daughter cells, both of which remain in the basal area until they undergo mitosis. The net result of symmetrical mitosis is an increase in the number of basal cells in the tissue. In the present study, reepithelialization of the DT with crypt cells prior to differentiation was a clear demonstration of symmetrical mitosis. It is not clear if symmetrical mitosis occurs in the normal cell renewal processes of the DT. It may be that a "status quo" is maintained between crypt cells and functional cells by asymmetric mitosis. In asymmetrical mitosis, an undifferentiated cell divides into two daughter cells one of which remains an undifferentiated cell, and the other matures into a non-dividing functional epithelial cell.

Insofar as the effects of ionizing radiation on oysters are concerned, many questions remain to be answered. For example, what are the effects on oyster survival and growth rates years after exposure to ionizing radiation? Also, are there any long-term histopathological changes in the tissues of irradiated oysters? Hopefully these questions may be answered through continuation of the studies discussed in this paper. The body weight/survival study is designed to continue for 3-5 years depending on the frequency of sampling. Through continuation of these studies it may be possible to compare the effects of some natural environmental stresses on irradiated and unirradiated oysters. It is also possible that the histopathological data that has been and will be collected can provide some insight into long-term effects of ionizing radiation on other tissues such as the gills and gonads, both thought to be

especially radiosensitive (Mix, 1976).

Other studies are required to assess the effect of age of the oyster at the time of irradiation on radiosensitivity. Research to determine the effect of environmental conditions such as temperature and salinity also need to be conducted. Also, radiotracer studies would be useful to ascertain the effects of irradiation on cell renewal systems and to determine what mechanisms lead to the restoration of radiation damaged tissues. For example, it may be possible using radiotracers to identify the origin of the small undifferentiated epithelial cells that were observed to repopulate the epithelial tissues of the stomach, gut, and CD. Other factors such as tissue oxygen tension, interphase chromosome volume, enzyme radiosensitivity, and DNA base composition may be important in evaluating the radiosensitivity observed in oysters.

The results of this study indicated that the juvenile Pacific oyster, C. gigas, is quite resistant to ionizing radiation. This radioresistance appears in part due to the surprisingly efficient cell renewal systems operating in the digestive tissues. Studies should be conducted to determine if other shellfish and if other invertebrate animals in general possess similar radioresistance.

## SUMMARY AND CONCLUSIONS

This study was designed to look at both the acute and long-term effects of ionizing radiation on Pacific oysters. Percent survival and changes in mean body weights were monitored for 238 days PI and oysters were sampled histologically for one year.

In the survival study, two periods of high mortality were observed. The first of these, termed the AL syndrome was seen 2-7 days PI in oysters receiving 75-200 krads. The second mortality period was observed from 40-60 days PI in oysters exposed to 10 krads or more and was referred to as the LTD syndrome.

The LD-50 dose for the Pacific oyster was found to be a function of time from the time of irradiation until approximately 80 days PI. The 238-day LD-50 dose was 16.5 krads.

The mean wet weight studies revealed that during the 238 days of the study, only oysters exposed to 20 krads or more weighed significantly less than the controls, although a dose dependent weight relationship was suggested in the 5 and 10-krad oysters. Also, although not statistically significant, oysters exposed to 500 and 1000 rads grew at a rate exceeding that of the controls from approximately 43 days until the termination of the experiment.

Histopathological degenerative syndromes were described in the digestive tissues of oysters exposed to both 16 and 40 krads. Oysters in both these groups exhibited degenerative changes that coincided with the LTD syndrome noted in the survival-wet weight study.

Tissue regeneration sequences were observed in the stomach, gut, CD, and DT of most oysters exposed to 16 krads and in a smaller number of oysters exposed to 40 krads. The earliest repair activities were observed in the DT's. Repopulation of the DT's in this study occurred similarly to that described by Mix and Sparks (1971). As in their study, repopulation of the DT was observed to begin with the formation of epithelial islands of crypt cells, which eventually proliferated and migrated around the tubule resulting in the tubule being repopulated with crypt cells. In the present study, following repopulation of DT's with crypt cells, differentiation of crypt cells into functional DT cells was observed.

Tissue recovery in the stomach, gut, and CD was observed and was believed to have been initiated by a previously undescribed cell population. Reepithelialization of these tissues was initiated by small undifferentiated basophilic cells. These cells were observed to eventually differentiate into normal appearing epithelial cells.

## REFERENCES CITED

- Baetcke, K. P., A. H. Sparrow, C. H. Nauman, and S. S. Schwemmer, 1967. The relationship of DNA content to nuclear and chromosome volumes and to radiosensitivity (LD<sub>50</sub>). Proceedings of the National Academy of Science, U. S. A. 58:533-540.
- Baptist, J. P., D. A. Wolfe, and D. R. Colby. 1976. Effects of chronic gamma radiation on the growth and survival of juvenile clams (Mercenaria mercenaria) and scallops (Argopecten irradians). Health Physics 30:79-83.
- Bergonié, J., and L. Tribondeau. 1959. Interpretation of some results of radiotherapy and an attempt at determining a logical technique of measurement (Translated by G. Fletcher). Radiation Research 11:587-588.
- Bullough, W. S. 1962. The control of mitotic activity in adult mammalian tissues. Biological Reviews 37:307-342.
- Burch, P. R. J., and R. G. Burwell. 1965. Self and not-self: a clonal induction approach to immunology. Quarterly Review of Biology 40:252-279.
- Casarett, A. P. 1968. Radiation Biology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 368 pp.
- Castor, L. N. 1971. Control of division by cell contact and serum concentration in cultures of 3T3 cells. Experimental Cell Research 68:17-24.
- Cheney, D. P. 1971. A summary of invertebrate leucocyte morphology with emphasis on blood elements of the Manila clam, Tapes semidecussata. Biological Bulletin 140:353-368.
- Cooley, J. L., and F. L. Miller, Jr. 1971. Effects of chronic irradiation on laboratory populations of the aquatic snail Physa heterostropha. Radiation Research 47:716-724.
- Cooley, J. L. 1973. Effects of chronic environmental radiation on a natural population of the aquatic snail Physa heterostropha. Radiation Research 54:130-140.
- deZwaan, A., and T. C. M. Wijsman. 1976. Anaerobic metabolism in bivalvia (mollusca): characteristics of anaerobic metabolism. Comparative biochemistry and Physiology 54B:313-324.

- DesVoigne, D. M., and A. K. Sparks. 1969. The reaction of the Pacific oyster, Crassostrea gigas, to homologous tissue implants. Journal of Invertebrate Pathology 14:293-300.
- Engel, D. W. 1967. Effect of single and continuous exposures of gamma irradiation on the survival and growth of the blue crab, Callinectes sapidus. Radiation Research 32(4):289-291.
- Engel, D. W. 1973. The radiation sensitivities of three species of fiddler crabs (Uca pugilator, U. pugnax, and U. minax). Chesapeake Science 14(4):289-291.
- Hargis, W. J., M. F. Arrighi, R. W. Ramsey, and R. Williams. 1957. Some effects of high frequency X-rays on the oyster drill Urosalpinx cinerea. Proceedings of the National Shellfisheries Association 47:68-72.
- Harmon, P. 1976. Preliminary summary of Co-60 calibration results. Progress report on OSU Co-60 calibration report submitted to Art Johnson, Radiation Safety Officer, OSU Radiation Center.
- Mix, M. C. 1970. The histopathological effects of ionizing radiation on the Pacific oyster, Crassostrea gigas. An examination of degenerative syndromes, cellular reparative mechanisms, and their relation to normal cell renewal systems. Ph.D. dissertation, University of Washington. 168 pp.
- Mix, M. C., and A. K. Sparks. 1970. Studies on the histopathological effects of ionizing radiation on the oyster, Crassostrea gigas. I. The degenerative phase involving digestive diverticula, stomach, and gut. Journal of Invertebrate Pathology 16:14-37.
- Mix, M. C. 1971. Cell renewal systems in the gut of the oyster, Crassostrea gigas. The Veliger 14(2):202-203.
- Mix, M. C., and A. K. Sparks. 1971. Repair of digestive tubule tissue of the Pacific oyster, Crassostrea gigas, damaged by ionizing radiation. Journal of Invertebrate Pathology 17:172-177.
- Mix, M. C. 1972. Chronic tissue degeneration in the Pacific oyster, Crassostrea gigas, following acute  $\gamma$ -irradiation. Radiation Research 49:176-189.
- Mix, M. C. 1976. A review of the histopathological effects of ionizing radiation on the Pacific oyster, Crassostrea gigas. Marine Fisheries Review 38(10):12-15.
- Okada, S. 1970. Radiation Biochemistry (Altman, K., G. Gerber and S. Okada, Eds.). Volume 1: Cells. Academic Press. New York, New York. 366 pp.



- Patt, H. M., and H. Quastler. 1963. Radiation effects on cell renewal and related systems. Physiological Reviews 43:357-396.
- Perlowagora-Szumlewicz, A. 1964. Survival, growth, and fecundity of Australorbis glabratus snails developed from eggs exposed to ionizing radiation. Experimental Parasitology 15:232-241.
- Price, T. J. 1965. Accumulation of radionuclides and the effects of radiation on molluscs. In: Public Health Service Publication Number 999-WP-25. pp. 202-210.
- Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. The American Journal of Hygiene 27:393-397.
- Rice, T. R., and J. P. Baptist. 1971. Ecological aspects of radioactivity in the marine environment. In: Environmental Radioactivity Symposium. Baltimore, Johns Hopkins Press. 145 pp.
- Sparrow, A. H., L. A. Schairer, and R. C. Sparrow. 1963. Relationship between nuclear volumes, chromosome numbers, and relative radiosensitivities. Science 141:163-166.
- Sparrow, A. H., A. G. Underbrink, and R. C. Sparrow. 1967. Chromosomes and cellular radiosensitivity. I. The relationship of  $D_0$  to chromosome volume and complexity in seventy-nine different organisms. Radiation Research 32:915-945.
- Steele, V. E., and C. S. Lange. 1976. Effects of irradiation on stem cell response to differentiation inhibitors in the planarian Dugesia etrusca. Radiation Research 67:21-29.
- Underbrink, A. G., A. H. Sparrow, and V. Pond. 1968. Chromosomes and cellular radiosensitivity. II. Use of the interrelationships among chromosome volume, nucleotide content, and  $D_0$  of 120 diverse organisms in predicting radiosensitivity. Radiation Botany 8:205-238.
- Westermarck, B. 1971. Proliferation control of cultivated human glia-like cells under "steady-state" conditions. Experimental Cell Research 69:259-264.
- White, J. C., and J. W. Angelovic. 1966. Tolerances of several marine species to Co 60 irradiation. Chesapeake Science 7(1):36-39.
- White, J. C., J. W. Angelovic, D. W. Engel, and E. M. David. 1967. Interactions of radiation, salinity, and temperature on estuarine organisms, pp. 29-35. In: Annual Report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, North Carolina. U. S. Fish and Wildlife Service, Circular 270.

## APPENDICES

Appendix A. Mortalities per group postirradiation.

Dose	Days Postirradiation																			
	2	3	5	7	14	21	30	35	43	48	55	62	72	77	84	91	98	113	141	167
0 rad																				
500 rad																				
1000 rad																				
5000 rad																				
10 krad										1		2		1						
20 krad									1	1	5	2	1	2						1
50 krad										2	5	9	1		1					
75 krad				2		1			1	2	6	4	1	1						
100 krad				3					3	6	6									
150 krad				10	1				1	3	2		1							
200 krad	4	10	4																	
0 rad																				

Appendix B. Mean wet weight per oyster postirradiation (in g).

Dose	Days Postirradiation													
	0	14	30	43	55	72	84	98	113	141	167	203	238 3/9/77	294 4/20/77
500 rad	10.8	14.0	21.4	22.1	25.4	30.3	35.7	39.4	43.4	54.2	57.3 ± 7.9	61.9 ± 8.5	68.6 ± 9.6	74.1 ± 10.5
1000 rad	10.1	12.6	19.1	19.5	23.1	27.8	32.9	37.2	41.5	50.3	53.5 ± 10.0	59.0 ± 10.3	62.9 ± 12.4	68.2 ± 11.9
5000 rad	10.7	13.3	18.4	19.4	21.9	26.5	30.9	32.2	36.2	41.0	47.7 ± 9.8	52.7 ± 8.9	57.9 ± 9.7	63.6 ± 9.6
10 krad	11.9	13.7	18.0	17.9	19.0	22.1	25.6	28.2	30.0	35.9	38.7 ± 8.3	41.1 ± 8.9	44.8 ± 9.7	50.4 ± 11.7
20 krad	12.1	13.2	16.1	15.1	15.2	15.1	18.1	18.7	19.3	20.9	21.7 ± 7.3	22.3 ± 8.1	24.3 ± 10.0	26.4 ± 12.4
50 krad	10.6	11.4	13.5	12.0	11.8	10.5								
75 krad	10.1	10.2	12.0	11.2	10.6	10.1								
100 krad	10.4	11.0	13.3	11.4										
150 krad	10.9	10.6	12.5	11.4	11.7									
200 krad	11.0													
0 rad	11.2	14.5	21.4	21.7	24.2	29.0	33.3	35.4	39.6	48.6	52.1 ± 10.0	56.5 ± 11.0	61.8 ± 12.3	64.5 ± 13.4

Appendix C. Mean length ( $\pm \sigma$ ) per oyster postirradiation (in cm).

Dose	Days Postirradiation																				
	26	34	41	48	55	61	68	83	96	110	126	131	138 n=20	155	167	181	196 n=30	225	250 n=20	286	321 n=10
200 R	1.78 ±.25	2.18 ±.31	2.78 ±.28	2.37 ±.39	3.37 ±.64	3.52 ±.46	3.65 ±.19	4.57 ±.49	4.36 ±1.32	5.89 ±.9	5.14 ±.56	ND	5.18 ±.65	5.30 ±.90	5.95 ±.63	6.88 ±.71	6.69 ±.90	ND	7.68 ±1.04	ND	8.10 ±1.21
600 R	1.46 ±.31	1.87 ±.12	2.29 ±.30	2.19 ±.31	2.76 ±.55	2.53 ±.58	3.00 ±.57	4.06 ±.33	4.76 ±.7	5.27 ±.49	5.43 ±.2	ND	4.96 ±.91	4.65 ±.37	6.06 ±1.13	5.65 ±1.12	5.96 ±.99	ND	6.85 ±1.07	ND	5.88 ±.96
1 kR	1.27 ±.10	1.69 ±.43	2.02 ±.22	2.16 ±.39	2.57 ±.28	2.71 ±.30	3.27 ±.65	3.77 ±.56	4.16 ±.54	5.05 ±.83	5.14 ±.94	ND	4.80 ±.82	5.63 ±.45	6.77 ±.47	4.79 ±.8	6.92 ±.94	ND	6.40 ±1.16	ND	6.82 ±1.01
2 kR	1.49 ±.11	1.72 ±.18	1.79 ±.23	2.17 ±.37	2.42 ±.37	2.78 ±.54	2.75 ±.39	3.78 ±.38	4.3 ±.87	4.88 ±.76	4.41 ±.66	ND	4.18 ±.73	4.6 ±.43	5.51 ±.60	5.15 ±.66	5.52 ±.81	ND	5.60 ±1.03	ND	5.79 ±1.18
5 kR	1.30 ±.22	1.25 ±.14	1.61 ±.06	1.78 ±.33	2.28 ±.29	1.74 ±.26	2.45 ±.5	3.24 ±.49	3.45 ±.86	4.77 ±.53	4.27 ±.75	ND	4.08 ±.87	4.93 ±.69	5.59 ±1.02	6.84 ±.28	6.58 ±.86	ND	7.10 ±1.12	ND	7.27 ±.98
8 krad	1.59 ±.15	1.48 ±.12	1.86 ±.17	2.33 ±.22	2.59 ±.18	2.63 ±.48	3.41 ±.66	3.18 ±.99	3.87 ±.47	4.77 ±.21	4.67 ±.73	ND	3.83 ±.55	4.13 ±.59	5.72 ±.29	5.41 ±.51	5.39 ±.69	ND	6.22 ±1.14	ND	6.7 ±1.19
16 krad	1.25 ±.37	1.27 ±.12	1.33 ±.15	1.31 ±.14	1.37 ±.21	1.79 ±.64	1.48 ±.33	2.04 ±.33	3.27 ±.52	3.48 ±.37	4.02 ±.24	ND	3.37 ±.67	3.4 ±.79	4.11 ±.6	4.72 ±.35	3.74 ±.62	ND	3.69 ±.65	ND	3.9 ±.51
40 krad	1.41 ±.10	1.13 ±.19	1.09 ±.04	1.23 ±.20	1.16 ±.26	.91 ±.25	1.08 ±.14	1.18 ±.19	1.27 ±.19	1.18 ±.25	1.34 ±.24	ND	1.11 ±.23	2.26 ±.21	1.97 ±.38	1.30 ±.08	1.72 ±.55	ND	--	ND	--
0 R control	1.42 ±.21	1.86 ±.29	2.28 ±.19	2.40 ±.36	2.79 ±.23	2.83 ±.42	2.99 ±.58	4.52 ±.96	4.75 ±.96	5.06 ±.36	5.66 ±.43	ND	5.13 ±.73	5.6 ±.8	5.84 ±.72	6.48 ±.76	6.99 ±.85	ND	7.08 ±.78	ND	7.3 ±.75

ND - no data.