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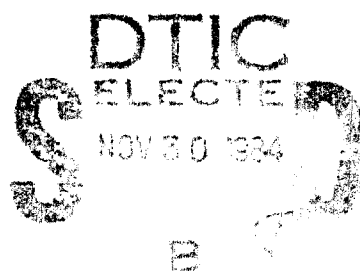


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Acute post-irradiation canine intestinal blood flow

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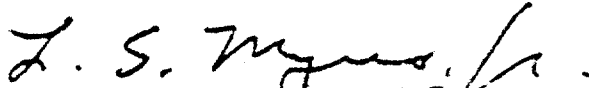
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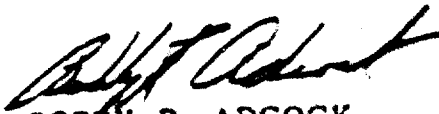
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20. ABSTRACT (continued)

blood flow was measured by the hydrogen polarographic technique, both before and after exposure to gamma radiation. Systemic blood pressures, blood gases and haematocrits were determined simultaneously. Data obtained from 12 sham-irradiated dogs and 12 irradiated dogs indicated that 90 Gy, whole-body, gamma radiation produced a 31 percent decrease in systemic mean blood pressure beginning within 20 min post-irradiation and lasting for at least 90 min. However, the intestinal submucosal blood flow did not decrease as anticipated, but it exhibited an actual postirradiation increase. This increase in postirradiation intestinal submucosal blood flow began within 5 min after irradiation and lasted for at least 90 min. Postirradiation haematocrits were 10.5 percent higher than those obtained before irradiation and those obtained from sham-irradiated subjects. Histopathological examination of ileal mucosa revealed significant pathologic lesions in some irradiated animals within two hours after exposure.

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Acute post-irradiation canine intestinal blood flow

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Radiation-induced early transient incapacitation (ETI) is accompanied by severe systemic hypotension, during which arterial blood pressure often decreases to less than 50 per cent of normal. One haemodynamic compensatory mechanism is increased peripheral resistance due to vasoconstriction. This vasoconstriction in the small intestine of dogs is disproportionately increased during haemorrhagic or endotoxic shock, and intestinal ischaemia is frequent. In an attempt to elucidate mechanisms underlying radiation-induced ETI and the gastrointestinal radiation syndrome, canine intestinal submucosal blood flow was measured by the hydrogen polarographic technique, both before and after exposure to gamma radiation. Systemic blood pressures, blood gases and haematocrits were determined simultaneously. Data obtained from 12 sham-irradiated dogs and 12 irradiated dogs indicated that 90 Gy, whole-body, gamma radiation produced a 31 per cent decrease in systemic mean blood pressure beginning within 20 min post-irradiation and lasting for at least 90 min. However, the intestinal submucosal blood flow did not decrease as anticipated, but it exhibited an actual post-irradiation increase. This increase in post-irradiation intestinal submucosal blood flow began within 5 min after irradiation and lasted for at least 90 min. Post-irradiation haematocrits were 10.5 per cent higher than those obtained before irradiation and those obtained from sham-irradiated subjects. Histopathological examination of ileal mucosa revealed significant pathologic lesions in some irradiated animals within two hours after exposure.

Indexing terms: intestinal blood flow, radiation shock, radiation pathology, radiation hypotension.

1. Introduction

Early transient incapacitation (ETI) is defined as a decrement in performance of a trained task, occurring transiently and within minutes of exposure to supralethal doses of ionizing irradiation (Carpenter 1979). Irradiation-induced hypotension has been implicated as the cause of ETI found with supralethal irradiation exposure (Miletich and Strike 1970). If blood pressure is reduced to a critical level, blood flow to vital organs may be reduced, resulting in an overall compromised performance and eventual incapacitation. However, post-irradiation hypotension does not occur with equal frequency in all species, having been reported in monkeys and rats but not in cats and dogs (Miletich and Strike 1970, Chaput *et al.* 1972, Pitchford 1968). Likewise, ETI was not seen in dogs following irradiation exposure of up to 400 Gy (Chaput *et al.* 1972, Pitchford 1968); however, a dose-related, progressive incapacitation was reported with those animals exposed to doses of 100-300 Gy generally experiencing partial incapacitation within minutes (Pitchford 1968). A recent,

unpublished, study by Bogo at the Armed Forces Radiobiology Research Institute has shown that the ED_{50} for performance decrement in rats to be approximately 100 Gy.

One possible explanation for ETI is a reduced cerebral oxygen supply resulting from a dramatic post-irradiation fall in blood pressure (Carpenter 1979). Since beagle dogs exhibit neither immediate post-irradiation hypotension nor ETI, cerebral ischaemia appears not to be present and the animals possess either a cardiovascular system that is insensitive to irradiation or a compensatory mechanism sufficient to maintain blood pressure. The latter seems to be the case since disproportionately increased splanchnic vasoconstriction is commonly observed in different types of shock (Abel and Murphy 1962).

To determine if the absence of immediate post-irradiation hypotension in dogs is due, in part, to a decreased blood flow to the small intestine, an experiment was designed to measure systemic blood pressure and intestinal submucosal blood flow immediately after exposure to supralethal doses of ionizing irradiation. Previous studies of post-irradiation intestinal blood flow in conjunction with systemic blood pressure reported changes occurring in 48–72 hours (Kabal *et al.* 1972a), but our effort was planned to measure both flow and pressure within 5–10 min following irradiation, therefore, irradiation dose of approximately 100 Gy was selected to achieve conditions occurring during periods of incapacitation and performance decrement previously reported (Chaput *et al.* 1972, Pitchford 1968). To investigate a possible relationship between irradiation, ischaemia and mucosal damage, histopathological examinations were made of the intestinal mucosa.

Understanding the physiological basis for irradiation-induced incapacitation and shock is essential to providing protection against these irradiation effects, for interpreting the effects of nuclear accidents, and for predicting the effects of nuclear warfare in order to prepare for casualty treatment.

2. Materials and methods

Twenty-four male beagles (Hazleton Research Animals, Cumberland, VA), 12–15 months old and weighing between 7.3 and 16.0 kg, were used in this study. The animals were divided randomly into a control group of 12 animals and a test group of 12 animals. Food was withheld from all dogs overnight before treatment, but water was available *ad libitum*. Research was conducted according to the principles enunciated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council.

Approximately three hours before irradiation or simulated irradiation, the animals were weighed and a foreleg shaved to facilitate administration of anesthetic by intravenous administration of 30 mg/kg sodium pentobarbital (Nembutal). Each dog was intubated with a cuffed endotracheal tube and respired using a forced volume respirator to maintain a stable blood pH and oxygen tension. After insertion of the endotracheal tube, each dog was placed in a supine position on a circulating water blanket to maintain body temperature. The water blanket and the dog were positioned in a standard wooden 'V' tray to maintain the animal in a steady supine position. With the animal in this position, a rectal probe was inserted to monitor body temperature during the experiment. A femoral arterial catheter was used to measure blood pressure, blood gas and haematocrit, while a femoral venous catheter

was used for maintenance doses of anaesthetic. The blood pressure was measured using a Statham P23 D6 pressure transducer.

For this experiment, the abdominal incision followed the linea alba from the umbilicus caudally to the pubic area. The incision was made with an electrocautery apparatus, additionally suturing any large bleeding vessels after clamping with haemostats. Once the midline incision was complete, the exposed gut was protected from drying and cooling by covering with a towel moistened with warm physiological saline.

A section of the small intestine approximately 15 cm from the ileocaecal junction was withdrawn from the abdomen in order to insert four electrodes (Teflon coated wire, 90 per cent platinum, 10 per cent iridium, 0.178 mm diameter, with a 3 mm uncoated tip) into the submucosa approximately 2 cm apart. After the electrodes were placed in the submucosa of the ileum and sewn in place with cotton sutures, they were connected to the hydrogen polarographic amplifier. The instrumented intestine was then returned to the peritoneal cavity and the abdominal wall sutured or clamped.

Intestinal submucosal blood flow was measured by the hydrogen clearance technique for 90 min before irradiation or sham irradiation and for 90 min after (Aukland *et al.* 1964, Young 1980). This technique is essentially an amperometric method, which measures the current induced in a platinum electrode by the reduction of hydrogen. The current produced has a linear relationship with the concentration of hydrogen in the tissue (Hyman 1961). Hydrogen was introduced into the blood via inhalation through the endotracheal tube at a rate of approximately 5 per cent of the normal respiratory rate for 1–2 min for each flow measurement. Blood flow was measured by each of the four electrodes approximately every 5 min. The electrodes were maintained electrically at +600 mV to reduce possible oxygen and ascorbate interference. The reference electrode, a stainless-steel needle, was placed in nearby tissue. Four recording electrodes were used to compensate for transient regional variations due to contractions, etc.

Current measurements from each electrode were fed through the polarographic amplifier, which produced curves depicting the clearance of hydrogen from the tissues. The clearance curves then analysed by a PDP 11-70 computer equipped with a VT55 terminal and a versatex plotter (Digital Equipment Corporation, Maynard, MA 01754). The computer operates in either real time or off line mode, using a FORTRAN IV-plus program written by AFRR's Computer Science Department. The first 60 s after the peak of the curves were neglected to obviate contamination by arterial recirculation (Martins *et al.* 1974). Data points were measured every second for 90 s. The flow was calculated between every two points and a linear regression performed over the 90 s period. Measurements from the four electrodes were then averaged to compensate for transient regional variations.

After 90 min of recording, the animals were disconnected from the respirator and recording apparatus to facilitate radiation. After irradiation, or sham irradiation for controls, the animals were immediately reconnected to the respirator and recording apparatus, and measurements were continued for a minimum of 90 min. Approximately 30 and 60 min pre- and post-irradiation, a blood sample was taken via the arterial catheter to determine pH and oxygen tension. Simultaneously, with blood gas determinations, haematocrit and temperature measurements were determined after radiation. After termination of the measurements, the electrodes were examined for verification of placement in the submucosal and then removed. At this

time representative tissue was removed from sections of the ileum between placement points of the electrodes. This tissue was placed in neutral 10 per cent buffered formalin and processed for histopathological examination. The animals were then euthanized with an i.v. injection of saturated MgSO_4 .

Data were grouped into 10 min intervals, measured in relation to midtime of irradiation, and plotted at the middle of the interval. The Wilcoxon Rank Sum Test was used to analyse statistically the blood pressure, blood flow, and haematocrit data. A 95 per cent level of confidence was employed to determine significance. Since all the animals were treated identically before irradiation or sham irradiation and since the pre-irradiation data for the control and test animals showed no significant difference, the pre-irradiation data for the irradiated and sham irradiated animals were combined.

Radiation was accomplished with a bilateral, whole-body, exposure to gamma ray photons from a ^{60}Co source located at AFRRRI. Exposure was limited to mean of 4.4 min at 22.59 Gy/min steady state, free-in-air. Dose rate measurements at depth were made with a tissue equivalent ionization chamber placed in a tube running along the midline of a beagle phantom. The measure midline intestinal dose rate was 20.19 Gy/min, giving a calculated total dose of 89.4 Gy.

Using hematoxylin and eosin stained sections, a series of histopathological examinations was performed on sections of the ileum. Initially, all the tissues were examined on a random basis without the knowledge of whether the dog was a control or test animal. All microscopic observations were recorded, including those interpreted as minor or insignificant. After the recording of all microscopic findings, the tissues were reexamined on a control and test basis.

3. Results

To keep variables to a minimum number, blood gases and blood pH were maintained at pre-irradiation levels by regulation of the volume and rate of the respirator. Temperature was maintained at the pre-irradiation level by using a circulating water blanket.

Post-irradiation mean arterial blood pressure (MAP) for the control of sham-irradiated animals remained fairly stable with only mild changes (figure 1). However,

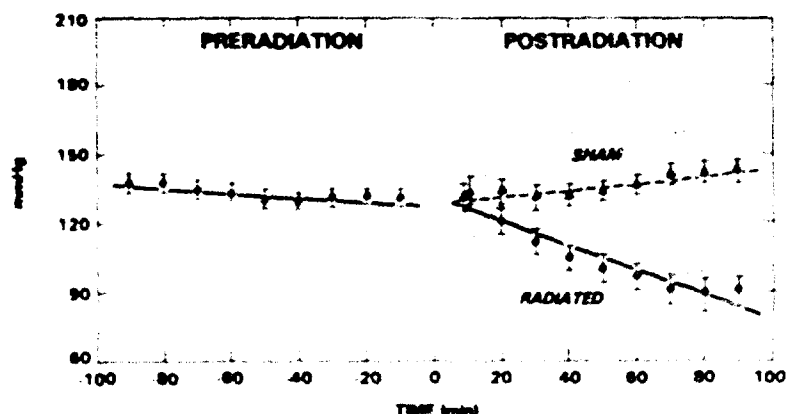


Figure 1. Mean arterial pressure 90 min before and 90 min after sham or real irradiation with 89 Gy, whole-body, gamma-irradiation. Error bars represent one standard error of the mean.

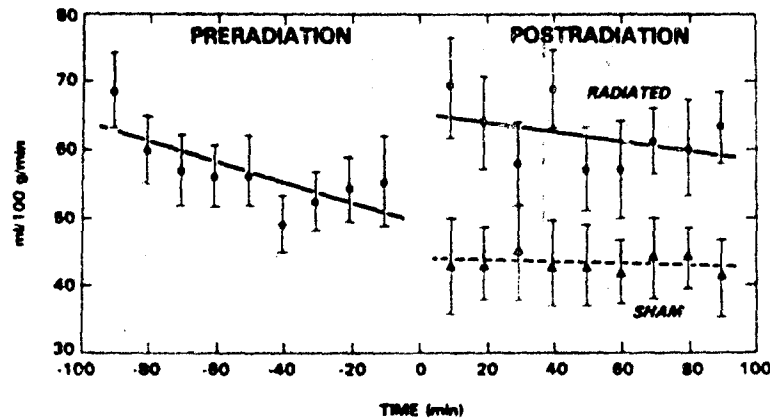


Figure 2. Mean intestinal blood flow 90 min before and 90 min after sham or real irradiation with 89 Gy, whole-body, gamma-irradiation. Error bars represent one standard error of the mean.

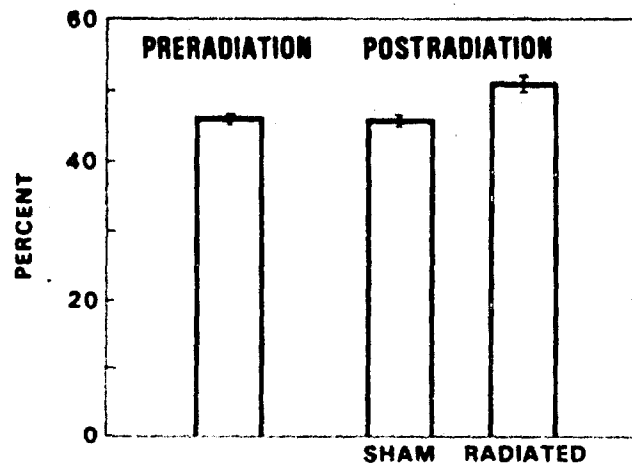


Figure 3. Mean haematocrits before and after sham or real irradiation with 89 Gy, whole-body, gamma-irradiation. Error bars represent one standard error of the mean.

within 20 min post-irradiation, the animals exposed to a midline dose of approximately 89 Gy began displaying a steady, rapid decrease in MAP. The drop in pressure reached its lowest point at 80 min post-irradiation, a level 35 per cent below the post-irradiation mean for un-irradiated animals as measured simultaneously. When the data are examined using the Wilcoxon Rank Sum Test, a significant difference is noted between the two groups, beginning at 50 min post-irradiation.

Both the control and irradiated animals showed a change in intestinal submucosal blood flow (IBF), compared to the levels the combined groups displayed before sham-irradiation or irradiation (figure 2). The IBF in the sham-irradiated or control group of dogs showed a decrease of 23 per cent, levelling off at a mean flow of approximately 43 ml of blood per 100 g of tissue per min. Meanwhile, the irradiated group exhibited an increase in blood flow to a mean level of 62 ml per 100 g of tissue per min, an approximate increase of 11 per cent over the pre-irradiation mean. A significant difference is seen between the IBF data from the irradiated dogs and the sham-irradiated dogs beginning at 10 min post-irradiation. The irradiated group had a significantly greater post-irradiation IBF than did the sham-irradiated group.

Haematocrits (HCT) of the sham-irradiated and irradiated animals exhibited a significant difference at the 95 per cent level of confidence. The mean HCT noted for the irradiated group was 50.6 while that for the sham-irradiated group was 45.8 (figure 3). The mean HCT for the pre-irradiation animals was 45.8, indicating no change for the sham-irradiated group but a significant 10.5 per cent post-irradiation increase for the irradiated group.

Light microscopic study of the ileal mucosa showed significant pathologic lesions in three of the animals receiving a midline exposure of approximately 89 Gy. In these dogs, distinct epithelial cell necrosis was present within the crypts of Lieberkuhn. This change was not evident in any of the control animals.

4. Discussion

This experiment was originally designed to determine if the absence of immediate post-irradiation hypotension in dogs was due, in part, to decreased blood flow in the small intestine. However, as clearly seen in the results, the animals in this experiment exhibited post-irradiated hypotension and increased intestinal submucosal blood flow.

The post-irradiation hypotension found in this experiment does not corroborate results reported by Kabal *et al.* (1972a, 1972b), who showed no significant differences in the mean arterial pressures of irradiated and non-irradiated animals prior to injection with either norepinephrine or isoproterenol at either 48 hours or 72 hours post-irradiation. This, of course, could be due to a difference of irradiation dose, technique and post-irradiation time of measurement. However, the hypotension reported here in dogs does resemble that seen by Miletich and Strike (Miletich and Strike 1970) in irradiated monkeys.

Kabal *et al.* (1972a) showed no significant differences between irradiated and non-irradiated intestinal blood flows until 72 hours post-irradiation. At this time the blood flow values for the irradiated group had decreased significantly. This present study, however, reports changes in intestinal submucosal blood flow (IBF) 10 min after irradiation. Additionally, the blood flow reported by Kabal was total intestinal blood flow and this study reports submucosal blood flow which contributes, along with mucosal blood flow, 65–92 per cent for the resting total intestinal blood flow in dogs (Granger *et al.* 1980). However, the true percentage of the total may be closer to 100 per cent since blood flow 2–5 mm from the recording electrode has been found to contribute significantly to the current of the electrode within the clearance time course (Young 1980), and the submucosa is less than 1 mm thick.

The sham-irradiated dogs showed a decrease in IBF with values well below that of the pre-irradiation group. The animals were fasted overnight and then maintained on anesthesia for approximately three hours prior to sham-irradiation, and this combination could be responsible for the IBF decrease before sham-irradiation and the levelling off after sham-irradiation at an apparent basal blood flow level of sufficient magnitude to maintain tissue metabolism (Granger *et al.* 1980).

The significant difference seen in haematocrits further suggests the presence of a chemical or humoral agent such as histamine in the intestinal circulation. Lee and Silverberg (1976) have shown that infusion of histamine into the small intestine not only causes an increase in intestinal blood flow but also produces a copious secretion of fluid following oedema. This loss of fluid from the blood would result in the observed increased haematocrit and contribute to post-irradiation hypotension.

The appearance of the ileal mucosa was consistent with previous reports of cellular irradiation damage to the small intestine (Quastler 1956, Quastler and Hampton 1962). Although the unusually large magnitude of exposure may be responsible for the appearance of cell damage in such a short post-irradiation interval, the short elapsed time may be the reason that only three of the 12 animals exhibited cell damage. However, ultrastructural examination of the crypt cells may reveal that damage did occur in a greater number of the animals.

A possible scenario for these results starts with the release of a chemical mediator from the irradiation-damaged crypt cells or mast cells. The chemical mediator would then cause an increase in vascular permeability and a vasodilation, possibly resulting in a decreased peripheral resistance to blood flow in the submucosa. The change in vascular permeability would result in fluid loss from the vascular system which, in turn, would contribute to both an increased haematocrit and a decreased systemic blood pressure. The decrease in submucosal peripheral resistance would allow an increased intestinal blood flow, contributing to systemic hypotension. Therefore, the post-irradiation increased intestinal blood flow, the systemic hypotension, and increased haematocrit may well be attributed to the irradiation-induced release of a mediator such as histamine. Indeed, histamine is stored in mast cells throughout the body and is released under the stimulus of X-irradiation (Carpenter 1979), and has been reported to cause intestinal vasodilation and increased intestinal blood flow in dogs (Granger *et al* 1980, Lee and Silverberg 1976, Shedadeh *et al.* 1969) and rats (Timmermans and Gerber 1980). Additionally, other data obtained indicate that 100 Gy, whole-body, gamma-irradiation produces an increase in plasma histamine levels (Cockerham *et al.* 1983). However, the findings of this paper, alone, are insufficient to support this connection.

Acknowledgments

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