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UNIVERSITY OF TORONTO

DEGREE FOR WHICH THESIS WAS PRESENTED Ph.D.

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UNIVERSITY OF TORONTO

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PROGRAM OF THE SENATE ORAL EXAMINATION

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

OF

WILLIAM MACGREGOR ROSS

10:00 a.m., Tuesday, May 11th, 1971

Room 108, 16 Hart House Circle

EFFECTS OF THERMAL STRESS AND GAMMA RADIATION ON MORTALITY AND HEMATOPOIESIS IN MICE

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Tight Binding

THESIS

EFFECTS OF THERMAL STRESS AND GAMMA RADIATION ON MORTALITY AND HEMATOPOIESIS IN MICE

(Summary)

Female mice (CBL albino) weighing 18 - 22 grams were exposed to two simulated components of a nuclear detonation, specifically the thermal component and the gamma component of the residual ionizing radiation. These were applied singly or in combination. The thermal stress (B) was delivered by a 500 watt I.R. bulb to the shaved back of the deeply anaesthetised animal, the exposed area representing about 10% of the body surface. The gamma component was delivered over a 96 hour period by a 250 Ci Co-60 source to the whole body either at a constant dose rate (CR), or at a fallout dose rate (F). The fallout rate was varied as a power function of time ... $R_t = R_{10} t^{-1.2}$. For combined stress experiments the thermal stress preceded the gamma radiation stress by 3.5 hours and was found to definitely modify mortality.

When a 30 second thermal exposure (B.30) preceded gamma radiation radioprotection was evident. The LD50 estimate was increased by about 16% over that for the radiation alone (e.g. 852 R to 1006 R). This definite protective effect was also attained when a 45 second exposure (B.45) was used. When a 15 second thermal exposure (B.15) preceded constant rate radiation the LD50 was decreased by 16%, but when it preceded fallout no significant change was noted. This latter effect would appear to be a result of differences in delivery of the stress (dose rate). It was found that mice succumbed more readily under a constant rate regime than a fallout regime.

Death distribution analysis revealed the protective effect of a 30 second thermal exposure upon irradiated mice to be manifested during the bone marrow syndrome. It was therefore proposed that the protective effect was exerted on the hematopoietic system and that an endogenous spleen colony assay might confirm this. Endogenous spleen colony counts were significantly higher in those animals subjected to 30 or 45 second thermal stress before constant rate irradiation than in those given a 15 second thermal stress before, or the irradiation alone. The D_0 values for the various burn treatment groups were the same (about 130 R) indicating no change in hematopoietic sensitivity was caused by varying

the severity of burn. Perhaps more stem cells were present at the time of irradiation or their efficiency at repopulation of the spleen was enhanced by the more severe burn. The dry weights of many spleens from each treatment group were recorded and found to reflect the mortality findings; a 30 second thermal stress protected mice from constant rate gamma radiation and a 15 second thermal stress enhanced radiation mortality.

Hypoxia as a possible mechanism was discussed as well as the role of bacterial endotoxins in radioprotection. The enhanced recovery of the hematopoietic system was apparently responsible for the reduction in mortality of severely burned mice during the bone marrow syndrome.

BIOGRAPHY

1941	Born, Toronto Canada
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Simulated Fallout and Thermal Trauma in Mice. 24th Technical sessions, Carleton Univ., Ottawa, Feb., 1970.

Simulated Fallout and Thermal Trauma in Mice. 4th International congress of Radiation Research, France, June 1970.

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EFFECTS OF THERMAL STRESS AND GAMMA RADIATION ON MORTALITY AND HEMATOPOIESIS IN MICE

by

WILLIAM MACGREGOR ROSS

DEPARTMENT OF ZOOLOGY

A Thesis submitted in conformity with the requirements for the Degree of Doctor of Philosophy in the University of Toronto

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INTRODUCTION

NUCLEAR WEAPONS

When a nuclear device is detonated in air the resultant energy is released as four separate components: blast and shock, 50% of the total energy; thermal radiation, 35%; initial ionizing radiation, 5%; and residual ionizing radiation or fallout, 10% (Glasstone, 1957).

The two components of primary interest in this study are the thermal and fallout components. The blast and shock and initial ionizing radiation cause massive injury thereby masking the more subtle effects of thermal and fallout radiation.

A thermal burn received down range from the detonation site varies from 1° - 3°, and the area exposed is dependent on clothing; a lightly-clad person would present a much larger area for burn than would a heavily-clad person. The average area exposed would be about 10% of the body surface, specifically in the head, neck and hand regions.

The exposure dose rate for gamma fallout radiation will increase with time as the activity of fallout material in the immediate vicinity increases. A peak is reached at 1 - ½ hours post-detonation when the rate of decay just balances the rate of deposition. Shortly, the dose rate declines, varying with time according to the relationship $R_t \propto t^{-m}$, where "R_t" is the dose rate in roentgens (R) per hour, "t" is the time post-detonation, and "m" is a constant determined

by the distribution of fission product species (Glasstone, 1957).

CELL RENEWAL

A cell renewal system may be considered a chain of cells in which any loss caused by either death or migration is countered quantitatively by the production of new cells. The system must have a self-sustaining reservoir of undifferentiated precursors or stem cells that sustain the level of the system at a steady rate (Pond et al, 1965).

Stem cells under normal conditions continually divide to produce one stem cell and one cell fated to differentiate into a specific mature functional cell (Doan, 1956). The latter daughter cell will divide once or several times before losing its capacity to divide, the dividing-maturing pool thus acting as a multiplier of a specific cell type. The cells then enter the maturing-only pool from which they pass into the mature functional pool. Cells become functional at a specific time which will depend upon the particular cell renewal system involved.

Disruption by radiation severely depletes the stem cell pool, those cells being the most radiosensitive. The mature functional cells are the most radioresistant. The more mature pools are depleted as they lose cells to the functional pool. Eventually even the functional pool suffers a decline in population of cells. The stem cell pool recovers gradually by homomorphogenic division, one stem cell dividing to form

two stem cells. When this pool has attained an adequate population of cells division becomes more heteromorphogenic, one stem cell dividing to form one stem cell and one cell destined for the functional pool. The more mature pools are now gradually replenished with cells as the cell renewal system begins to return to normal operation. If repair is not sufficient and death ensues, then the mortality syndrome that appears will be characteristic of the cell system that failed to repair itself.

ACUTE RADIATION SYNDROME

The appearance of a syndrome is principally due to the failure of a particular organ or organ system. This occurs when the number of functional cells in the cell renewal system falls below a critical value. However, radiation damages the entire animal and the clinical picture reflects all changes. This is most evident during the transition from one syndrome to the next when there is an admixture of symptoms. The time limits of all syndromes stated below are arbitrary estimates and there is some overlap.

The central nervous system syndrome is caused by damage to the functional cells of the brain, death ensuing within minutes or hours. Since these are non-proliferating cells, this syndrome is seen only at doses sufficiently high (above 10 kR) to cause serious damage to the radioresistant mature functional cells present during irradiation. The symptoms include convulsions, severe hypotension and shock (Langham,

et al, 1956).

The gastrointestinal syndrome is caused by damage to the small bowel epithelium, death ensuing within five days. The intestinal stem cells in the crypts and the dividing-maturing cells are relatively sensitive to radiation. The mechanism of acute intestinal radiation death has four phases. Irradiation blocks the production of viable cells to replace those normally sloughed off the villi, with a resultant depletion of intestinal epithelial cells. This denudation and breakdown of the barrier separating the lumen from the interior of the body leads to death unless the lining is replaced (Quastler, 1956; Quastler and Lucker, 1959).

The bacteremic syndrome, sometimes called H. II, results from the intestinal damage, death ensuing between days 5 and 7. But bacteria invade the circulatory system through the denuded epithelium of the villi causing infection (bacteremia) throughout the body. Animals may also succumb in this period to late gastrointestinal or early bone marrow failure (Cronkhite and Tyler, 1963; Bond et al, 1965; Miller et al, 1951).

The bone marrow syndrome results from hematopoietic damage, death ensuing from days 9 - 30. Stem cells are sensitive to radiation, their numbers and capacity to proliferate greatly reduced by only a few hundred roentgens. The dividing and maturing pool is less sensitive but is also markedly reduced in size by sub-lethal doses. Blood elements are depleted in a matter of days as mature, functional cells are removed at a normal rate and not replaced. Extensive granulo-

cytopenia allows infection to develop, thrombopenia results in hemorrhage, and erythropenia leads to anemia. All these secondary consequences may lead to death, but virtually all deaths occur prior to days 20 to 25 (Bond et al, 1960).

The causes of the syndromes just described have been identified by various methods. The use of preventive measures such as the shielding of sensitive organs during irradiation has been most instructive. By shielding the entire abdomen or the exteriorized small intestine, the gastrointestinal syndrome does not appear. If the long bones are shielded the bone marrow syndrome does not develop. The technique of shielding a specific organ while the rest of the body is irradiated identifies the syndrome caused by damage to that organ (Quastler and Sucker, 1956; Austin et al, 1956).

Another approach is to consider the treatment (therapy) which alleviates the symptoms. The administration of antibiotics such as streptomycin eliminates the bacteremic syndrome, indicating the cause of such deaths to be infection (Baxter et al, 1953). The bone marrow syndrome may be reduced or negated by the administration of a sufficiently large concentration of healthy bone marrow suspended in saline. The stem cells from the transplanted marrow assume the function of hemopoiesis in a lethally irradiated host. The spleen accepts these viable stem cells as it would normally accept the host's own cells and colonies are formed, each representing one viable stem cell (exogenous spleen colonies). With lower doses endogenous spleen

colonies and formed from the host's own stem cells (McCulloch and Till, 1961).

Control Factors

Various factors have been found to influence the response to radiation. Dose is the primary one, but for sparsely ionizing (low LET) x-ray radiation several other factors may also be important. Some of these are oxygen tension, dose rate and thermal stress. Increased oxygen tension has been found to increase the susceptibility of cells to lethal damage (Gray and Alexander, 1961; Puro, 1966), whereas anoxia exerts a protective effect (Lajtha and Oliver, 1961). A decrease in dose rate over the lower range of values is also known to decrease the amount of damage (Gray and Alexander, 1961; Puro, 1966). Thermal burn has been shown to exert a synergistic effect on irradiated animals (Fraser et al, 1965; Allen and Sheline, 1964), or, with more extensive exposures and chronic dose rates, a protective effect (Hoar, 1967). Allen and Sheline (1964) studied the effect of burn by determining the LD50, the dose that must be exceeded to cause 50% mortality. The LD50 decreased with increasing dose.

Many such factors could be and are of prime importance in the treatment of tumours by radiation, and, more basically in the field of tumour research toward an understanding of the manner in which radiation affects living tissue. Protraction of exposure through either fractionation or protraction of the [illegible] exposure [illegible] as discussed in

radiotherapy. If a means could be found of making tumour tissue more sensitive to radiation then less radiation would need to be applied to achieve the same degree of damage to the tissue. This would result in less damage to neighbouring tissues since the dose would have been decreased.

PURPOSE OF THIS RESEARCH PROJECT

Preliminary findings demonstrated a protective effect of thermal stress when this preceded various dosages of gamma radiation delivered under a simulated fallout regime (Ross, 1967). The present study was undertaken to further confirm and expand the above results employing various thermal exposures combined with varying dose rate deliveries of the gamma component (fallout and constant rate). Consideration would be given to the syndromes contributing to mortality over a 30 day period.

METHODS AND MATERIALS

ANIMALS

All animals used in the following experiments were CBL female albino mice (Canadian Breeding Laboratories Ltd., P. Q.) weighing 18 - 22 grams. The CBL strain is a randomly outbred stock originating from the Swiss-Webster albino strain, Charles River. A randomly outbred stock was chosen because the effects observed following stress treatment should more closely represent the effects for a general population exposed to a nuclear detonation than a pure strain would. However, the sex of irradiated animals affects radiosensitivity quite markedly, so only one sex (female) was employed. Males are generally more radiosensitive than females (Chapman, 1955). The animals were shipped from Montreal via air express and truck, and were housed on arrival in a temporary quarantine room outside the radiation facility (Ramsay Wright Zoological Laboratories, University of Toronto). Six mice were housed in each metal cage with fine wood chip bedding. Purina mouse chow (Purina Mills, Toronto) and tap water (chlorinated and fluoridated) were provided ad libitum. A constant atmosphere was maintained in the animal facility (73° F, 55% relative humidity) where the animals were kept post treatment. The light cycle was set for 12 hours of light and 12 hours of darkness.

PRELIMINARY TREATMENT

Two days were allowed for the animals to equilibrate from possible stresses induced by transportation and handling, following which they were weighed for experimentation. Those within the acceptable limits, 18 - 22 grams, were randomly distributed to experimental groups. Those groups slated to receive a thermal exposure were given 0.025 - 0.030 cc. of pentobarbitone-sodium anaesthetic intraperitonealy through the abdominal wall between the ventral mid-line and the right hind leg. The majority of the mice weighed between 20 and 21 grams and the amount injected was not varied; the main consideration was deep anaesthesia. The effects of anaesthesia had worn off by the beginning of irradiation. The anaesthetic was prepared by dissolving 300 mgm. of the dry powder (British Drug Houses) in 5 cc. sterile mammalian saline solution (Baxter Laboratories of Canada Ltd.). The hypodermic syringe and needle were pre-sterilized by dry heat, and both needle and injection area were swabbed with 70% ethanol prior to each injection. When the animal was in deep anaesthesia, the back was shaved as cleanly as possible by "Oster" electric clippers fitted with a #40 surgical head.

CONTROL STRESS

Several control experiments were performed throughout the time course of this project. One group of animals was only weighed and not stressed in any other way, a second group was anaesthetised and shaved, and a third group was anaesthetised, shaved and subjected to thermal stress. This last group, designated "B", would provide control mortality

values for groups subjected to both thermal stress and gamma radiation stress.

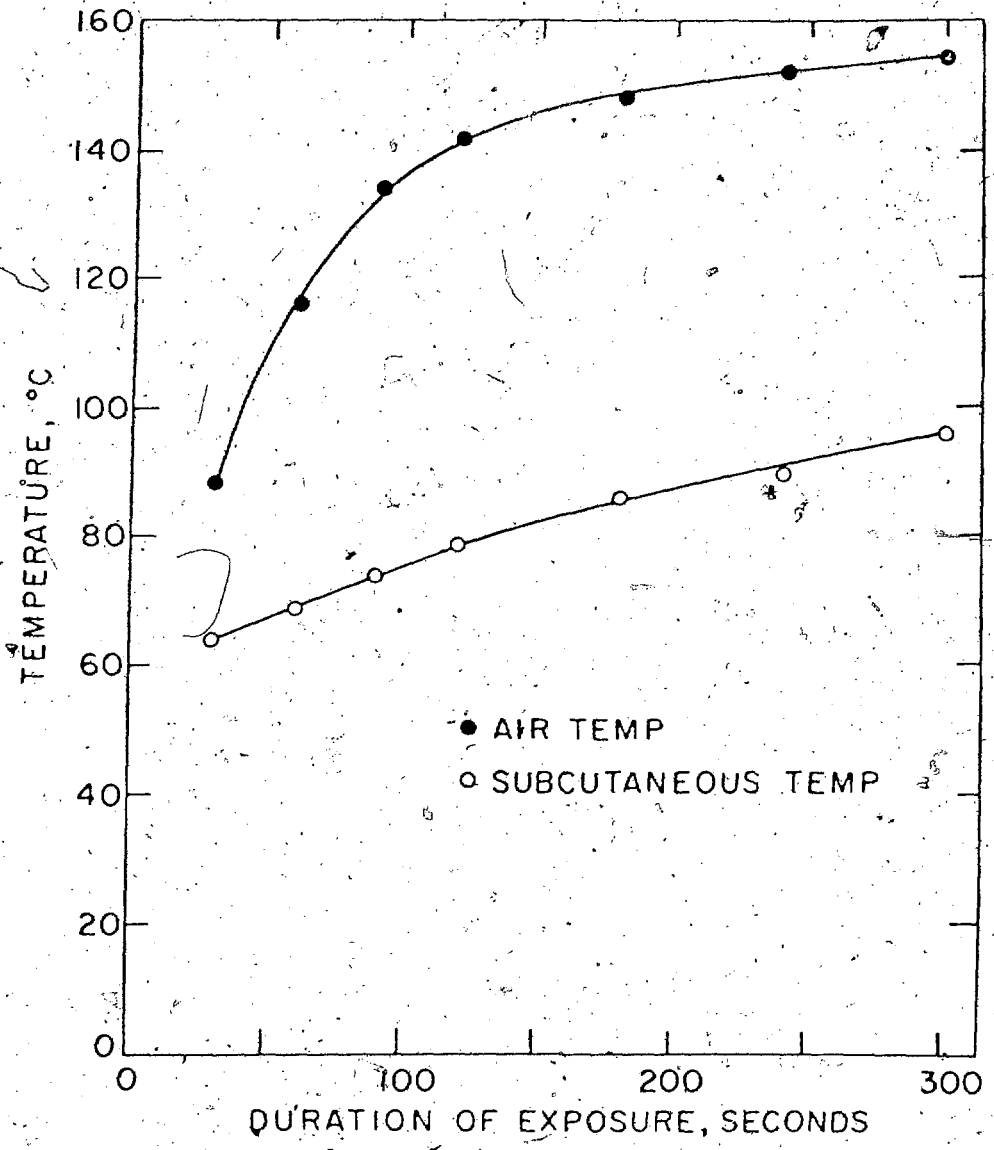
THERMAL STRESS

The thermal component of a simulated nuclear detonation in air (Glasstone, 1957) was delivered by means of a General Electric 500 watt industrial infrared lamp (500 G30/i) mounted inside a sheet metal container of dimensions 25 cm. x 25 cm. x 25 cm. (Fig. 1). The bulb was aligned 5 mm. below a 1 cm. x 4 cm. opening cut out of the top of the box. The animal to be stressed would be placed over this exposure opening for varying lengths of time. The top of the box was cooled for animal handling by passing cold water through a fine copper coil 5 mm. in diameter. This coil was covered by a sheet of metal and a layer of asbestos paper. The lamp was allowed to warm up for 30 min. prior to every experiment, since it had been found that the thermal output of the lamp reached a plateau at this time. The subcutaneous temperature was measured using a sensitive thermometer over an extended period of time and was found to attain a value of 64° C after a 30 second exposure (Fig. 2). This is sufficient to cause protein coagulation and necrosis of the epithelium, indicative of a 2° burn (Brooks et al, 1952).

The anaesthetised, shaved animal was placed on the thermal stress unit so that the shaved area was situated directly over the 1 cm. x 4 cm. opening and 0.5 cm. above the surface of the

Figure 1: Thermal Stress Unit.
The 500 watt infrared bulb is approximately 0.5 cm. below the 1 cm. x 4 cm. opening in the top of the box. The cooling coil under the asbestos sheet is connected to a source of cold water by plastic tubing.

Figure 2: Temperature Responses.
Responses are shown in air and subcutaneously. After 30 seconds, a subcutaneous temperature of 64° C is achieved. This is sufficient to coagulate protein and thus cause necrosis, indicative of 2° burn.


160
140
120
100
80
60
40
20
0
TEMPERATURE, °C
0
100
200
300
DURATION OF EXPOSURE, SECONDS
AIR TEMP
SUBCUTANEOUS TEMP

lamp. This would allow an area approximating 10% of the total body surface to be burned. Exposure times varied from 15 seconds to 45 seconds; such exposures would result in thermal burns ranging from 1° to 3° respectively (Catena, 1967). Following exposure the animals were returned to their cages and observed for 30 days.

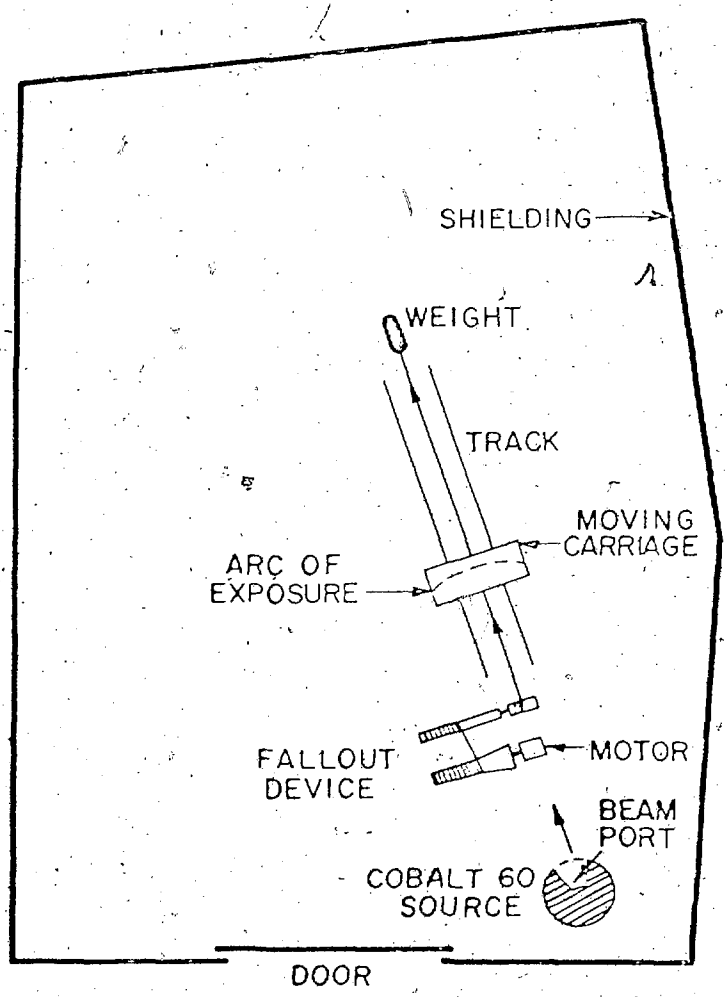
GAMMA RADIATION STRESS

The gamma component of the residual ionizing radiation in an early fallout field was simulated in the laboratory by employing a fixed source of 250 Ci of ^{60}Co housed in a "Gamma-Beam 150 C Cobalt Irradiator" supplied by the Commercial Products Division of Atomic Energy of Canada Ltd. (Fig. 3). The beamport head of this unit allows a field of exposure of approximately 1.05 metres x 0.90 metres at a distance of one metre from the centreline of the source.

The radiation was delivered at a fallout dose rate (F) or at a constant dose rate (CR). For a fallout experiment the polycarbonate cages were placed on a plexiglass trolley on an arc of exposure so all cages would be equidistant from the source. The trolley could run along two tracks down a shallow incline away from the source, being constrained by a cable from a gear ratio device (Fig. 4). This device consisted of a shaft rotating at a constant rate of one rotation every two hours which turned a conical gear with a helical groove. The length of cable unwound from this groove

Figure 3: Radiation Facility.
Looking towards the control room the Cobalt-60 source is housed within the large white leaded steel castle. The plexiglass trolley for animal cages is shown on the track in the foreground. Refer to Figure 4 for a fallout facility schematic.

Figure 4: Fallout Facility Schematic.
The trolley moves down the inclined tracks away from the Cobalt-60 source. The weight at the end of the tracks maintains a steady pull on the trolley as the gear unwinds the cable in the gear ratio (fallout) device.

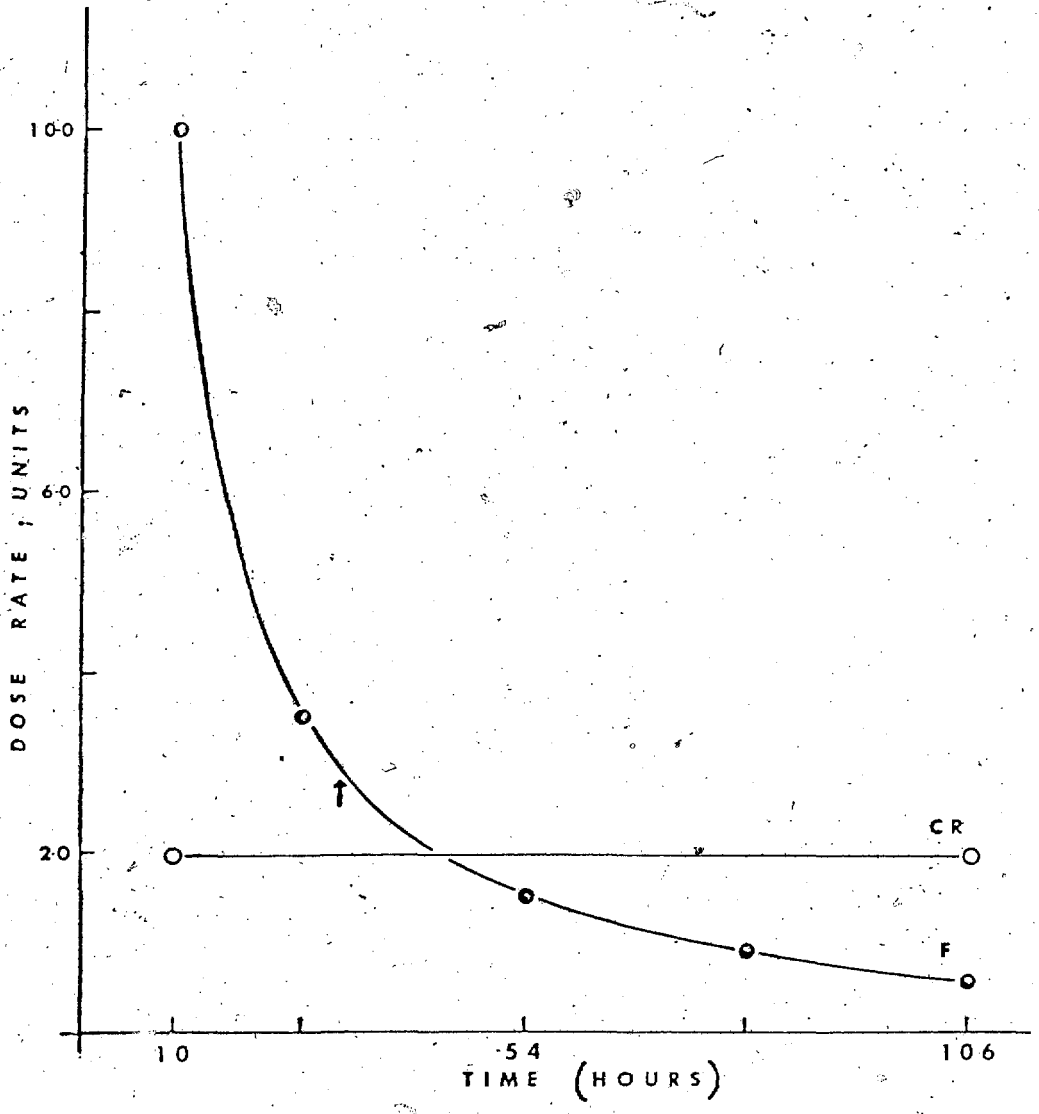


FALLOUT FACILITY

decreased with each successive revolution as the radius decreased, and this caused the dose rate delivered to the animals on the trolley to vary as a power function of time, where $R_t = R_{10} t^{-1.2}$ (Baker et al, 1961). This formula actually holds from 3 hours to 120 hours post detonation (t3 - t120), according to Glasstone (1957). In the present experiments the fallout was assumed to arrive 10 hours post detonation and to continue for an effective period of 96 hours, that is from 10 hours to 106 hours post detonation (t10 - t106).

For a constant rate experiment the dose rate was held constant for the 96 hour period instead of declining exponentially as with fallout, and the cages were placed on stationary tables. To obtain the same total dose under CR as under F, a much lower starting dose rate was required. A fallout delivery began at a very high dose rate declining rapidly to a very low dose rate. Half the total dose under fallout was delivered in the first 24 hours at dose rates much higher than the constant rate; the dose rates for the remainder of the 96 hour period were generally much lower than the constant rate. Figure 5 gives a comparison of the dose rates for fallout and constant rate, the dose rate in arbitrary units being plotted against time. The constant rate is 20% of the starting fallout rate, while the final fallout rate is 6% of the starting fallout rate and 33% of the constant rate. Starting dose rates under fallout varied from 0.421 - 4.452 R/minute and under constant rate from 0.132 - 0.968 R/minute.

Figure 5: Fallout and Constant Rate Delivery.
A comparison of the dose rates over the 96 hour irradiation period for fallout delivery and constant rate delivery. The constant rate is always 20% of the starting dose rate of fallout, and half the total dose of fallout is delivered in the first 28 hours (indicated by the arrow). The final dose rate under fallout is approximately 6% of the starting dose rate, and 33% of the constant rate.


10·0
6·0
2·0
DOSE RATE ; UNITS
CR
F
10
·54
1·06
TIME (HOURS)

The animals destined for gamma radiation were allocated to cages on the trolley or tables, 12 per cage. It had previously been found that no difference was evident between groups that were anaesthetised and shaved prior to irradiation and groups that were not (Ross, 1967). The cages were aligned on an arc of exposure, following which the radiation room was vacated and sealed. The source was then raised by remote control into the exposed position for the 96 hour period. Once a day during this period the source was lowered into the safe position and the cages were checked for mortality, food and water. The cages were interchanged daily and the arc of exposure on which they were situated was checked and altered so that each cage remained equidistant from the source. The arc became more shallow as the trolley proceded away from the source.

Following irradiation the animals were removed from the cages and placed in the smaller metal animal colony cages, 6 per cage, for the duration of the 30 day period of study. The care, bedding, food, and water bottle were changed once a week. After 30 days all survivors were disposed of by administration of an overdose of ether.

COMBINED TREATMENT

When the two stresses were combined, mice were placed in irradiation cages immediately after burning; the thermal stress preceded the gamma radiation stress by 3.5 - 4 hours. This particular fractionation time was used because it was

found to be the average time required for the animals to recover from deep anaesthesia. By waiting until the animals were at least beginning to display definite signs of recovery, it was assured that no anaesthetic deaths would be recorded as stress mortalities. Those animals that had succumbed to the anaesthetic were removed prior to the beginning of the run. The anaesthetic deaths amounted to less than 1% of the total number of animals allocated for each experiment.

The thermal stress groups served as controls for the combined runs. The thermal mortality was applied as a control correction factor so the "actual" effect of combining the two stresses would become apparent. From the corrected data the LD^{50}_{30} estimates were calculated for the various treatments and employed as a parameter in assessing the sensitivity of each treatment.

PROBIT ANALYSIS

The probit transformation is a method of converting a quantal sigmoid response curve into a linear response which can be more easily analysed. The constant "5" is added to the normal deviate to eliminate negative values, and the resultant quantity is a probit ("probability-unit"). A plot of probit mortality allows the examination of sample normality; a linear trend would be indicative of normally-distributed data.

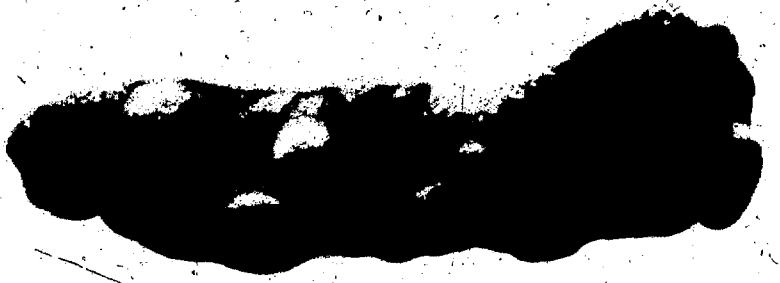
Probit linear regressions are often weighted because

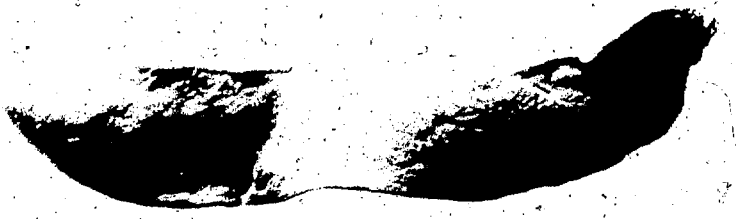
values about the LD50 are the most accurate, the accuracy declining as the extremes are approached. Between probit values of 4 and 6 the data is heavily weighted, but beyond these values substantially less weight is applied. The weighted linear regressions for these experiments were calculated by the method of least squares together with the 95% confidence limits of the LD50 estimates (Reid, 1969).

ENDOGENOUS SPLEEN COLONIES

An assay was performed to discover whether the observed mortality results were a reflection of activity in the hematopoietic system. This entailed the sacrifice by cervical dislocation of several animals from each treatment group on day 12, the removal of the spleens, and the fixing of the spleens in Bouin's Solution (British Drug Houses). After a minimum wait of 24 hours all colonies over 0.5 mm. diameter, easily discernible as pale yellow spots on the darker brown spleen (Fig. 6), were counted. The data so accumulated were treated by the $\log(x + 1)$ transformation after the method of Smith et al (1966). This transformation allows spleens with zero colonies to be included in the data analysis, whereas a log (x) transformation would eliminate them since there is no log value for zero. The number of colonies recorded for each spleen was increased by one, the new values converted to logarithms (base 10), and a mean determined. Then the antilog of the mean was decreased by one to give the actual geometric

Figure 6: Endogenous Spleen Colonies.
These spleens have been killed and fixed in Bouin's Solution. The upper spleen received a dose of 700 R of constant rate gamma radiation; the lower one received 900 R. There are several distinct colonies present on the 700 R spleen but none on the 900 R one. The spleen receiving less radiation is also larger.





median.

The spleens were later heat-dried to a constant weight for 36 hours at 100° F and weighed in batches (cage lots) of six to obtain the splenic dry weights.

DOSIMETRY

All dosimetry was performed with the Baldwin-Farmer Mk. II Substandard Dosemeter with a lucite build-up cap (wall thickness, 4.8 mm.) over a thimble ionization chamber. The lucite build-up cap was used to ensure "electronic equilibrium". The probe was placed in a polycarbonate irradiation cage on millet to give maximum backscatter conditions as would be experienced if bedding and several mice were present in the cage. Dosimetry was checked frequently because of constant source decay, approximately 1% per month. Several readings were taken at one metre from the centre-line of the source and a mean value was obtained. The dose rate was calculated and corrected for barometric pressure and temperature and for the inherent error of the probe itself.

For the fallout runs the limited number of gears available restricted the number of possible total dosages that could be employed. However the source was steadily decaying so several different dosages could be applied over the project period with each gear. The gear ratio used for a particular run determined the starting distance from the source. For example, a gear ratio of 1.25 : 1.00 would require a starting distance

of 1.25 metres from the source. The total dosage for the 96 hour treatment period was calculated by measuring the starting dose rate at 1.25 metres, applying to it the appropriate corrections for barometric pressure, temperature and the dosemeter, and multiplying this corrected dose rate by the mathematical constant of the gear ratio device (Baker et al, 1961).

For constant rate runs, the dose rate was measured at one metre and the distance required for the animals to receive the desired dose was calculated by the inverse square law.

Calibration of the probe was against a 20 mCi ^{90}Sr source (SI 22) which accompanied the dosemeter. The correction factor obtained for the B.F.K. II instrument was 0.99 during the fallout runs, but the replacement probe employed during the constant rate runs had a correction factor of 1.05 (National Physical Laboratory, London, England).

Dosimetry has been quoted in terms of Roentgens (R). The dosage in R was matched by means of a lithium fluoride (LiF) dosimeter surgically inserted into the abdominal cavity of a mouse.

SYNDROME DELINEATION

For the current project, the generally accepted syndrome periods for acute irradiation were altered to suit the chronic (96 hour) irradiation. Because of the duration of the delivery, "chronological day 0" was not acceptable as "syndrome day 0". It was decided that "syndrome day 0" should be approximately

at the midpoint of the irradiation, about 24 - 48 hours after the onset of irradiation. This designation was supported by the observed peaks in the mortality data. The syndromes have arbitrary time limits with no clear cut distinction between them; there is an overlap of symptoms. In the present study the acute irradiation syndrome periods had to be varied to suit the chronic dose delivery. The gastrointestinal syndrome was considered to occur within the first 6 days rather than the first 5 days. The bacteremic syndrome occurred between days 7 and 11 instead of 5 and 8. The bone marrow syndrome occurred between days 12 and 30 instead of 10 and 30, and predominately between days 12 and 20.

RESULTS

PARAMETERS

Several parameters were employed to assess the damage created by thermal stress, gamma radiation stress and combinations of these.

The total 30 day mortality was used to obtain an LD_{30}^{50} estimate for each type of treatment; this would be the dose required to produce 50% lethality in a stressed population in 30 days.

Daily mortality records were kept and from this data the cumulative mortality over the 30 day period could be studied with the different treatments. A death distribution analysis by time period or syndrome was made and the mean survival time of decedents determined for each treatment. The time interval between thermal stress and gamma radiation was varied in a series of experiments to determine if there was an optimal interval for mortality effects.

An endogenous spleen colony assay was conducted to assess damage to the hematopoietic or blood cell forming system. The dry weights of several spleens were recorded as an additional parameter of hematopoietic damage.

TOTAL MORTALITY DATA

Control Stress

Several control experiments were performed to determine the

effect on mortality of the various treatments applied.

One set of controls was not treated in any way aside from being weighed and placed in polycarbonate cages as if to be irradiated ("sham irradiation"). A second set of controls was anaesthetised and shaved only, not being stressed in any other way. A third set was anaesthetised, shaved and exposed to various thermal exposures (15 - 45 seconds). The first two sets showed no apparent effects from the treatments with respect to mortality. However, the third set exhibited increasing mortality with increasing exposure time. This set will be further discussed in the following section.

Thermal Stress

The mortality data from the thermal stress experiments, a 15 second burn (B.15), a 30 second burn (B.30) and a 45 second burn (B.45), is recorded in Table 2 (see page 33 for zero dose and 15, 30 and 45 second burn). The greater the duration of exposure, and thence the degree of burn, the higher the mortality, ranging from 2.1% after a 15 second exposure to 11.5% after a 45 second exposure. The variable number of animals is simply due to the variable number of experiments performed for each exposure during the project, the results of which were pooled. The mortality data for the three thermal exposures studied displays a linear relationship with exposure time as seen in Figure 7. A typical thermally-stressed mouse on day 4 post burn is shown in Figure 8; the exposure time was 30 seconds (B.30). The position and area of

Figure 7: Thermal Stress Mortality Response for Mice. Exposures varied from 15 - 47 seconds, giving a linear response for 30-day mortality.

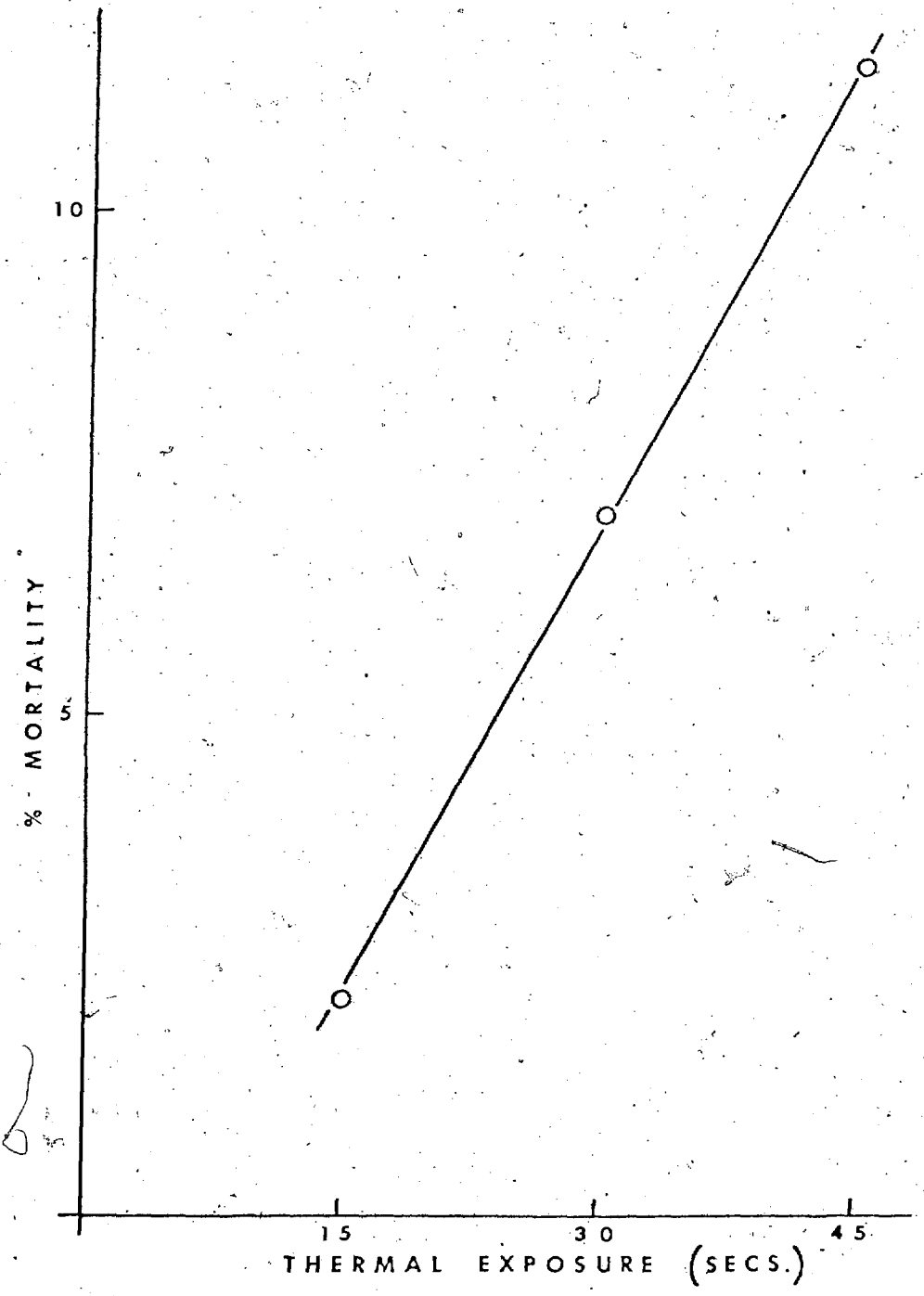

10
5
% - MORTALITY
15
30
45
THERMAL EXPOSURE (SECS.)

Figure 8: Thermally-stressed Mouse (Day 4).
This mouse was given a 30 second exposure. The burned area represents approximately 10% of the body surface area, and is identified by the presence of necrosis.

of the burned tissue within the shaved area is readily identified by the presence of necrosis.

Fallout Stress

The total percent mortality by day 30 for animals subjected to fallout irradiation alone is recorded in Table 1. The number of animals irradiated at each dosage was a sufficiently large enough sample population for a mortality study; the minimum number used was 30. A number of control runs were done simultaneously and their results pooled but no mortality was observed. The doses varied from 500 R to 5030 R, the LD^{0}_{30} estimated to be about 500 R and the LD^{100}_{30} about 1500 R. When the mortality data are plotted (Fig. 9), a typical sigmoid dose response curve appears. From 600 R to 1300 R the response is essentially linear, the curve then approaching 0% and 100% mortality asymptotically.

Combined Stress (Burn + Fallout)

When the thermal and fallout stresses were combined the resultant mortality was considered to be caused by the radiation but mediated by the thermal stress. Three possible actions can account for the observed mortality: mortality from the thermal burn alone; mortality from the fallout radiation alone; and mortality from an interaction of burn and radiation. By subtracting the thermal stress mortality from the combined stress mortality, the adjusted mortality thus obtained should more accurately reflect the true extent of the interaction of the two stresses.

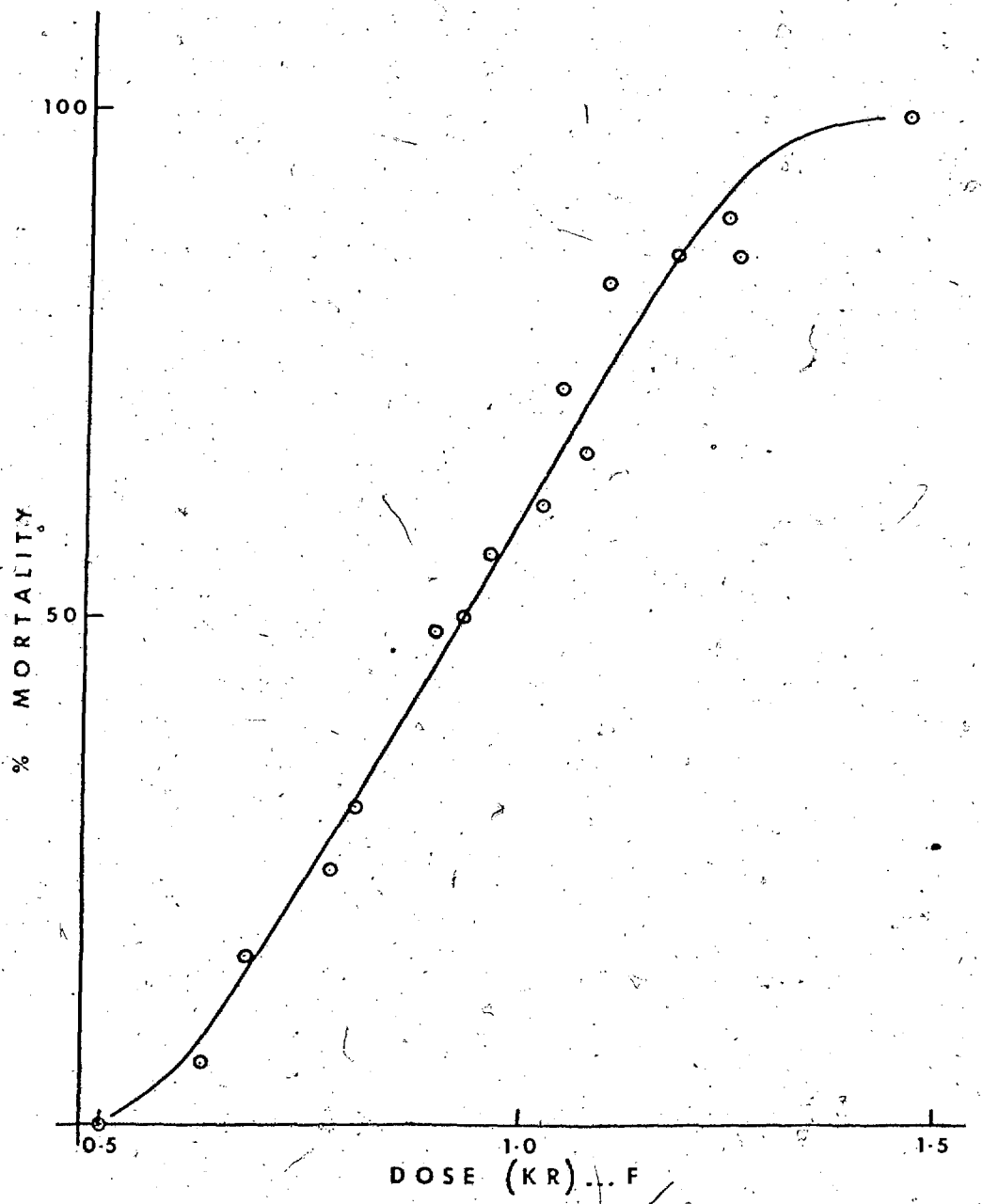
TABLE 1

THIRTY DAY MORTALITY - FALLOUT STRESS

DOSE (R)	LOG DOSE	ANIMALS USED	PERCENT MORTALITY	PROBIT MORTALITY
CONTROL	---	138	0	---*
500	2.6990	30	0	---
620	2.7924	66	6.1	3.4536
670	2.8261	36	16.7	4.0339
772	2.8876	36	25.0	4.3255
800	2.9031	48	31.3	4.5126
890	2.9494	35	48.6	4.9649
925	2.9661	48	50.0	5.0000
955	2.9800	48	56.3	5.1586
1020	3.0086	36	61.1	5.2819
1044	3.0187	48	72.9	5.6098
1070	3.0294	36	66.7	5.4316
1096	3.0398	36	83.3	5.9661
1180	3.0719	36	86.1	6.0848
1240	3.0934	30	90.0	6.2816
1256	3.0990	50	86.0	6.0803
1460	3.1644	36	100	---
1550	3.1903	36	100	---
1700	3.2305	30	100	---
2300	3.3617	34	100	---
2600	3.4150	30	100	---
3700	3.5682	30	100	---
5030	3.7016	30	100	---

* No probit value for 0% and 100% mortality

Figure 9: Sigmoidal Mortality Response for Mice (Fallout Stress) Various total dosages were delivered over a 96 hour period (t10 - t106). The values shown are for mortality over a 30 day period post-exposure (kR = 1000 R).


100
50
% MORTALITY
0·5
1·0
1·5
DOSE (KR)

The adjusted percent mortality for combined stress is recorded in Table 2. There is a consistent trend toward greater mortality as the fallout dosage is increased, but the 30 second burn combinations exhibit mortality that is markedly reduced from that of fallout alone. This protective effect is quite definite and consistent over the range of dosages studied although less marked for some experiments than for others. The 15 second burn combinations display only a small deviation from fallout alone doses. The 45 second burn combination show a protective effect approximately equal in magnitude to that of 30 second burn combinations.

The preceding observations can be graphically illustrated by a probit transformation of the mortality data from Tables 1 and 2. The probit of percent mortality is plotted against the logarithm of the dose (Figure 10). A 15 second burn affords only slight protection to fallout stressed mice, but a 30 second burn affords marked protection. The weighted linear regressions, calculated and fitted by the method of least squares (Reid, 1969), are essentially parallel as indicated by their slopes; 8.77 for fallout, 8.83 for B.15 + F and 9.78 for B.30 + F (Table 3).

The LD_{30}^{50} estimates clearly support the protective effect exerted by 30 and 45 second thermal stress upon fallout radiated mice (Table 3). A considerably greater amount of fallout radiation would thus be required to achieve 50% lethality in thermally pre-stressed (B.30) mice than in mice

TABLE 2

THIRTY DAY MORTALITY - COMBINED STRESS (BURN + FALLOUT)

BURN (SEC)	DOSE (R)	LOG DOSE	ANIMALS USED	PERCENT* MORTALITY	PROBIT MORTALITY
CONTROL		---	138	0	---
15	0	1.1761	141	2.1	2.9665
15	800	2.9031	48	29.2	4.4524
15	955	2.9800	48	50.0	5.0000
15	1044	3.0187	50	61.9	5.3029
15	1256	3.0990	46	89.2	6.2372
30	0	1.4771	313	7.0	3.5242
30	475	2.6767	48	0	---
30	800	2.9031	48	20.1	4.1619
30	925	2.9661	48	24.3	4.3033
30	955	2.9800	50	27.0	4.3872
30	1044	3.0187	48	55.5	5.1383
30	1070	3.0294	36	68.0	5.4677
30	1180	3.0719	35	64.4	5.3692
30	1256	3.0990	48	80.5	5.8596
45	0	1.653	130	11.5	3.7996
45	800	2.9031	22	11.2	3.7840
45	955	2.9800	24	9.3	3.6775
45	1044	3.0187	24	59.3	5.2353
45	1256	3.0990	24	84.3	6.0069

* Percent mortality is adjusted; burn alone mortality has been subtracted.

Figure 10: Probit Mortality Curves for Mice; Fallout Treatments. The values shown are for mouse mortality over a 30 day period post-exposure. The slopes and LD50 estimates for each treatment are listed in Table 3.

Code: closed circles, fallout (F)
open circles, 15 sec. burn plus fallout (B.15 + F)
open triangles, 30 sec. burn plus fallout (B.30 + F)

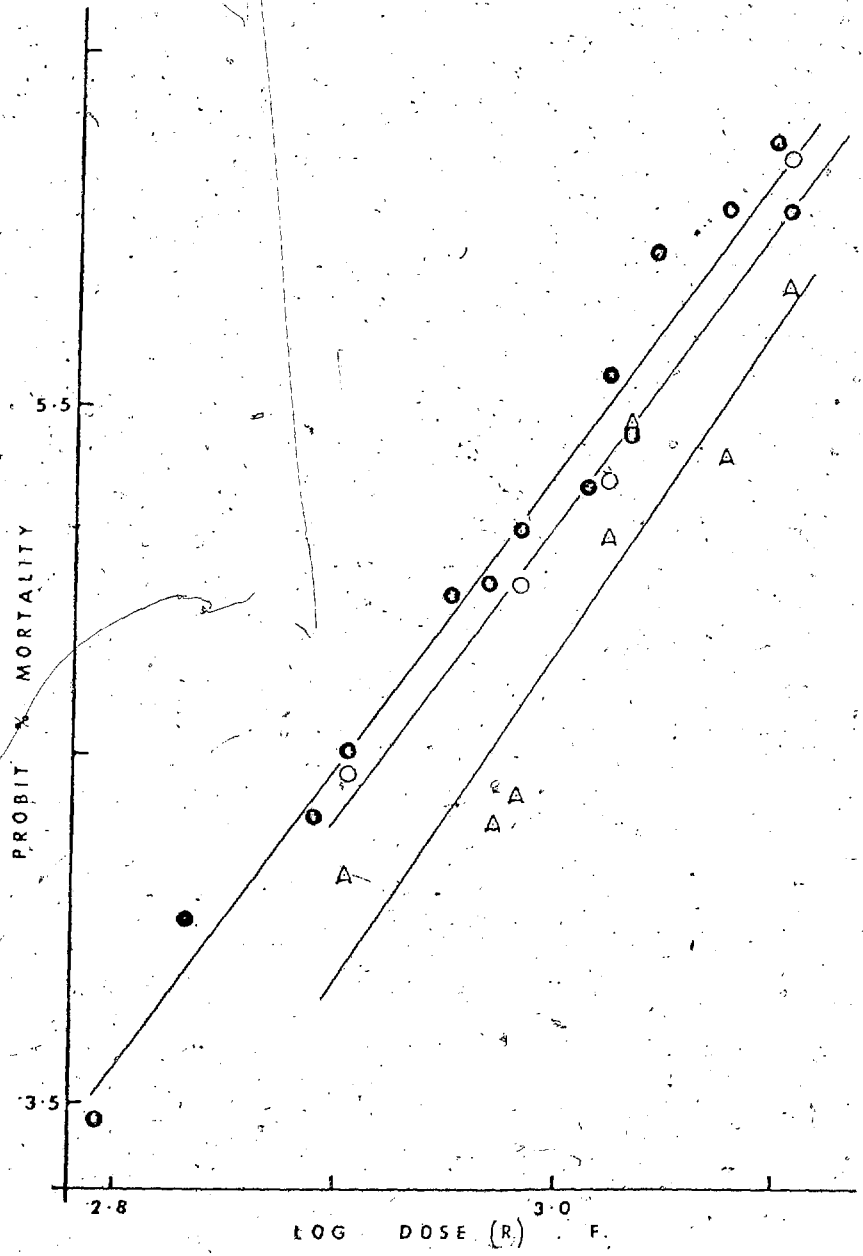

5·5
3·5
PROBIT MORTALITY
2·8
3·0
LOG DOSE (R)
F.

TABLE 3

LD_{30}^{50} ESTIMATES FROM PROBIT ANALYSIS - FALLOUT REGIMES

RADIATION REGIME	LD_{30}^{50} (R)	95% CONFIDENCE LIMITS	RELATIVE EFFECT	REGRESSION SLOPE
FALLOUT	911	897 - 925	1.00	8.77
B.15 + F	942	918 - 967	1.03	8.83
B.30 + F	1038	1019 - 1056	1.14	9.78
B.45 + F	1011	976 - 1047	1.11	11.41

Note: All estimates of the LD50 and of the regression line differ significantly ($P < 0.05$) among regimes.

receiving only the radiation. The necessary increase in fallout radiation is 14% (from 911 R to 1038 R) as shown by the relative effect (relative to the LD50 for fallout alone), so protection is conclusive. The 95% confidence limits indicate that this is significant.

The combination of a 45 second burn with 800 R of fallout radiation resulted in a mortality that is out of line with the regression fitted to the other three doses, and was therefore deleted from the calculation of the weighted regression.

Constant Rate Stress

The preceding results show a definite protective effect to manifest when B.30 or B.45 is combined with fallout radiation. It was assumed that if the dose were delivered at a constant rate instead of as simulated fallout the effects would be the same. With constant rate delivery the experiments would not be affected by the decay of the ^{60}Co source or limited by the specific gear ratios available. A few doses could therefore be repeated several times and the results pooled. Such a large population at each dose should increase the statistical validity of the pooled mortality results. In Table 4, it can be seen that from 760 R to 1300 R under constant rate delivery there are many more animals than at comparable fallout doses (Table 1).

As a comparison of fallout and constant rate radiation consider that 670 R of fallout cause 16.7% mortality, while 700 R at a constant rate cause 26.6%; also, 1240 R of fallout

TABLE 4

THIRTY DAY MORTALITY - CONSTANT RATE STRESS

DOSE (R)	LOG DOSE	ANIMALS USED	PERCENT MORTALITY	PROBIT MORTALITY
CONTROL	----	138	0	----
700	2.8451	233	26.6	4.3750
900	2.9542	208	55.8	5.1459
1100	3.0414	213	80.8	5.8705
1300	3.1139	97	92.8	6.4611
1800	3.2553	48	100	----
2400	3.3802	47	100	----
2500	3.3979	48	100	----
3000	3.4771	48	100	----
3450	3.5378	48	100	----
4000	3.6021	48	100	----
5000	3.6990	81	100	----

cause 60.0% mortality, and 1300 R at a constant rate results in 92.8%. Many of the 1300 R runs were eliminated from the pool for probit analysis since they experienced 100% lethality. These data cannot be assigned a probit value, nor can 0% lethality.

Combined Stress (Burn + Constant Rate)

A 15 second burn combined with a constant rate dosage (B.15 + CR) causes a much greater mortality than constant rate (CR) alone (Table 5), as opposed to negligible protection by B.15 + F over fallout alone. Only one run with B.15 + 1300 R treatment showed survival so only that run could be used for probit analysis. Although this meant a considerably smaller number of animals yet it should be remembered that this point, being near an extreme of mortality, is given considerably less weight than those points nearer the LD50.

A 30 second burn combined with constant rate radiation (B.30 + CR) causes a very definite protective effect over the entire lethal range as compared with the constant rate alone. This is similar to the result observed with B.30 + F.

The B.45 + CR treatment has a protective effect also, equivalent in degree to that of B.30 + CR. This is quite similar to the effect of B.45 + F. With B.45 + 700 R (CR) the mortality was out of line with a regression fitted to the other three doses and was therefore deleted from the calculation of the weighted linear regression for B.45 + CR.

The relationship of the various combined stress regimes to

TABLE 5

THIRTY DAY MORTALITY - COMBINED STRESS (BURN + CONSTANT RATE)

BURN (SEC) +	DOSE (R)	LOG DOSE	ANIMALS USED	PERCENT* MORTALITY	PROBIT MORTALITY
CONTROL		----	138	0	----
15	0	1.1761	141	2.1	2.9665
15	700	2.8451	95	46.3	4.9071
15	900	2.9542	113	76.7	5.7290
15	1100	3.0414	118	88.6	6.2055
15	1300	3.1139	22	93.4	6.5063
30	0	1.4771	313	7.0	3.5242
30	700	2.8451	119	7.3	3.5462
30	900	2.9542	123	37.7	4.6866
30	1100	3.0414	93	61.8	5.3002
30	1300	3.1139	72	81.9	5.9116
45	0	1.6532	130	11.5	3.7996
45	700	2.8451	123	22.6	4.2479
45	900	2.9542	148	36.5	4.6549
45	1100	3.0414	120	66.8	5.4344
45	1300	3.1139	72	78.8	5.7995

* Adjusted as in Table 2

constant rate radiation alone is graphically illustrated by a probit plot (Fig. 11). The pertinent observations to be made here are that the 15 second burn plus constant rate treatment definitely increases the mortality over that of constant rate radiation alone, and the 30 second burn plus constant rate treatment definitely decreases the mortality. There appears to be some convergence of the three regressions towards the higher doses, as evidenced visually and by comparison of the slopes in the regression formulae. However, the deviation in slope is not very great and the lines are essentially parallel within the range of dosages employed. The slopes are 7.59 for constant rate doses, 6.49 for B.15 + CR and 8.58 for B.30 + CR.

The LD^{50}_{30} estimates as calculated from the probit regression formulae for the various treatment groups support the synergistic effect of B.15 + CR over CR and the protective effect of B.30 + CR and B.45 + CR over CR (Table 6). The superficial burn (B.15) causes a higher mortality in irradiated mice than if no burn had been applied; the lethal dose to half a population in 30 days is decreased by 16% (from 852 R to 713 R) when mice are prestressed with a 15 second thermal exposure.

The severe burn (B.30) causes a lower mortality in irradiated mice than if no burn had been applied; the lethal dose to half a population in 30 days is increased by 18% (from 852 R to 1006 R) when mice are prestressed with a 30 second thermal exposure. Both the synergistic and protective

Figure 11: Probit Mortality Curves for Mice; Constant Rate Treatments.
The values shown are for mortality over a 30 day period post-exposure. The LD50 estimates are listed in Table 6.

Code: closed circles, constant rate (CR)
open circles, 15 sec. thermal stress plus constant rate (B.15 + CR)
open triangles, 30 sec. thermal stress plus constant rate (B.30 + CR)

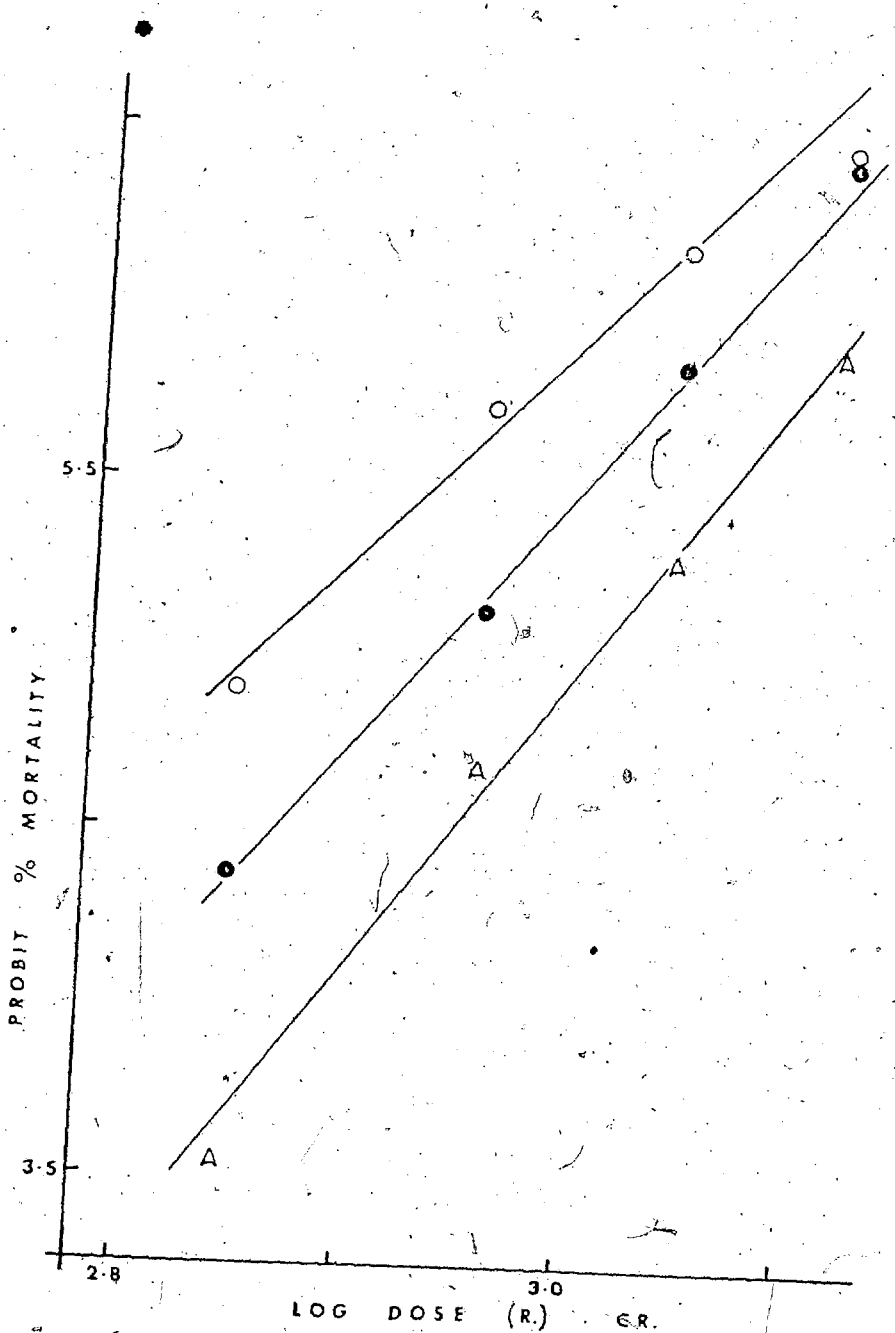

5·5
3·5
PROBIT % MORTALITY
2·8
3·0
LOG DOSE (R.) E.R.
O
O
O
A
A
A

TABLE 6

LD^{50}_{30} ESTIMATES FROM PROBIT ANALYSIS - CONSTANT RATE REGIMES

RADIATION REGIME	LD^{50}_{30} (R)	95% CONFIDENCE LIMITS	RELATIVE EFFECT	REGRESSION SLOPE
CONSTANT RATE	852	838 - 866	1.00	7.59
3.15 + CR	7[illegible]	685 - 742	0.84	6.49
3.30 + CR	1006	986 - 1025	1.18	8.58
3.45 + CR	9[illegible]0	968 - 1012	1.16	7.61

Note: All estimates of the LD50 and of the regression line differ significantly ($P < 0.05$) among regimes.

effects are significant on the basis of 95% confidence limits.

Fallout and constant rate radiation treatments are compared on the basis of LD50 and probit regression slope in Table 7. All the constant rate treatments have a lower LD50 estimate and a lesser slope than the comparable fallout treatments. The combined stress groups are essentially similar in effect except for the 15 second combinations. When a 15 second burn is combined with fallout the mortality is slightly lower than that for fallout alone, but with constant rate radiation it is definitely synergistic, the mortality being markedly greater than that for constant rate radiation alone.

Fallout is less damaging than constant rate radiation to mice on the basis of the LD50 values. The LD50 estimate for fallout is 911 R $\pm$ 14 R (95% confidence limits) while for constant rate radiation is 852 R $\pm$ 14 R. The 95% confidence limits indicate that the 7% difference in LD50 estimates is significant.

This difference is graphically illustrated on the probit plot (Fig. 12). The linear regressions are separated, but converge as the LD_{30}^{100} is approached.

TIME-MORTALITY DATA

Cumulative Mortality

The daily mortality (cumulative) for the various treatments involving fallout, constant rate and thermal stress is displayed

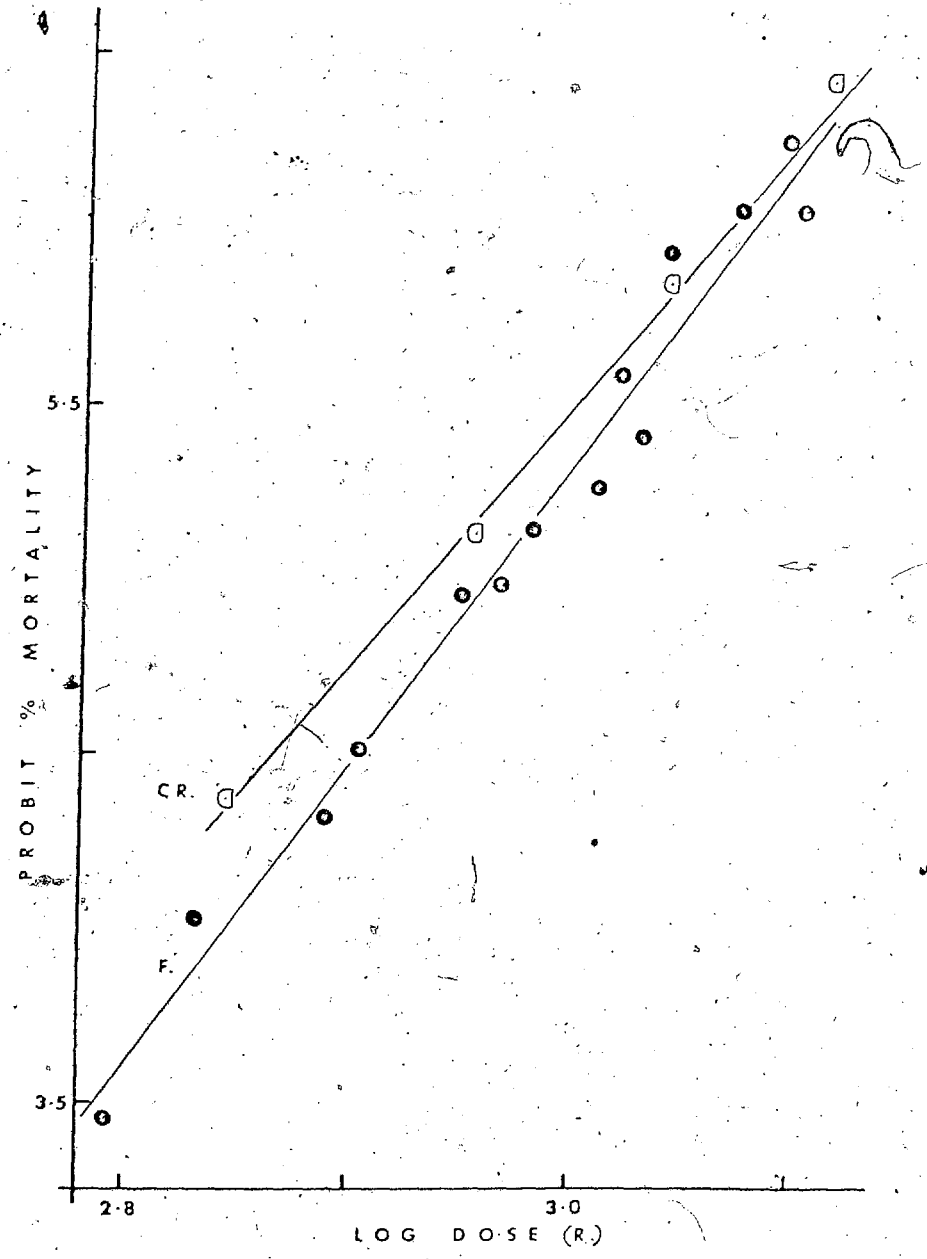
TABLE 7

A COMPARISON OF LD50 VALUES FOR FALLOUT AND CONSTANT RATE REGIMES

RADIATION REGIME	LD_{30}^{50} (R)	95% CONFIDENCE LIMITS	REGRESSION SLOPE
FALLOUT	911	897 - 925	8.77
CONSTANT RATE	852	838 - 866	7.59
B.15 + F	942	918 - 967	8.83
B.15 + CR	713	685 - 742	6.49
B.30 + F	1038	1019 - 1056	9.78
B.30 + CR	1006	986 - 1025	8.58
B.45 + F	1011	976 - 1047	11.41
B.45 + CR	990	968 - 1013	7.61

Figure 12: Probit Mortality Curves For Mice; Fallout versus Constant Rate.
The values shown are for mortality over a 30 day period post-exposure. The LD50 estimates are compared in Table 7.

Code: closed circles, fallout (F)
open circles, constant rate (CR)


5·5
3·5
PROBIT % MORTALITY
C.R.
F.
2·8
3·0
LOG DOSE (R.)

in Figures 13 - 17. The results of several fallout doses from 62 R to 5000 R appear in Figure 14. An increase in dose results in an increase in the rate of mortality as well as in the mortality accumulated by day 30. Deaths also occur earlier at higher doses, mostly in the 12 - 30 day period (bone marrow syndrome) over the lethal range. After a supralethal dose of 5000 R most deaths occur in the 7 - 11 day period and after 1000 R in the 0 - 6 day period (gastrointestinal syndrome). The latter dose actually causes death between days 5 and 6; generally, death does not occur within the first three days until doses are attained capable of causing extensive central nervous system damage (CNS syndrome). Deaths within the first 48 hours may be caused by thermal stress which results in neurogenic and cardiovascular shock.

The thermal stress exposures varied from 0.1 second exposure (3.1 cal/cm^2) to 2.4 second exposure (8.4 cal/cm^2). As exposure time is increased, the mortality increases and shows an earlier onset. During the main period of lethality the dose response is fairly linear (Fig. 14).

The two stresses burn (.5 cal/cm^2) when combined with fallout exhibits a higher mortality than does fallout alone over the first two weeks, but in the latter half of the thirty day period exhibits a lower mortality, resulting in an overall protective effect at day 30. This reduced mortality is particularly evident during the bone marrow phase. In contrast, the .5 burn seems to exert very little influence on the mortality

Figure 13: Percent Mortality Cumulative (30 Days); Fallout Stress (F).
All deaths recorded are expressed by the accumulated percent mortality by the day on which the deaths occurred.

The dose represented by each curve is indicated on the graph.

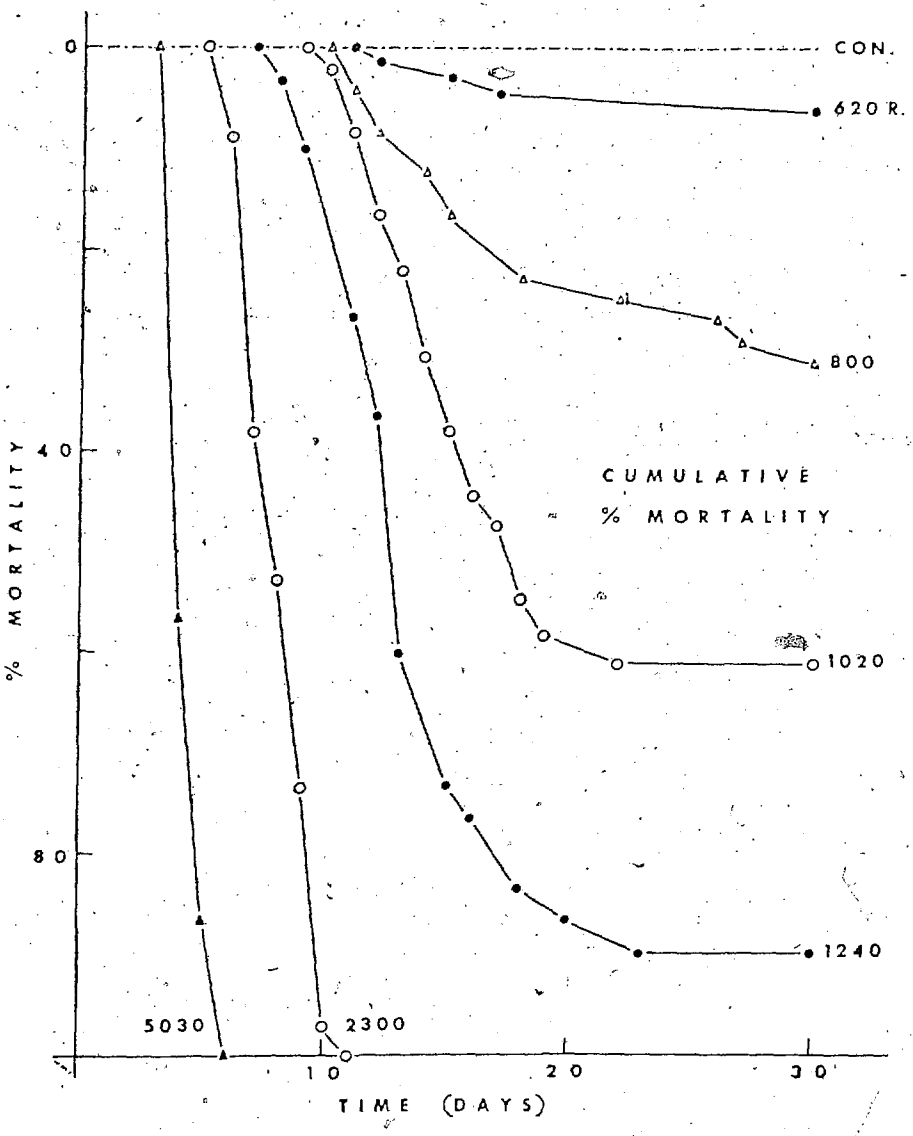
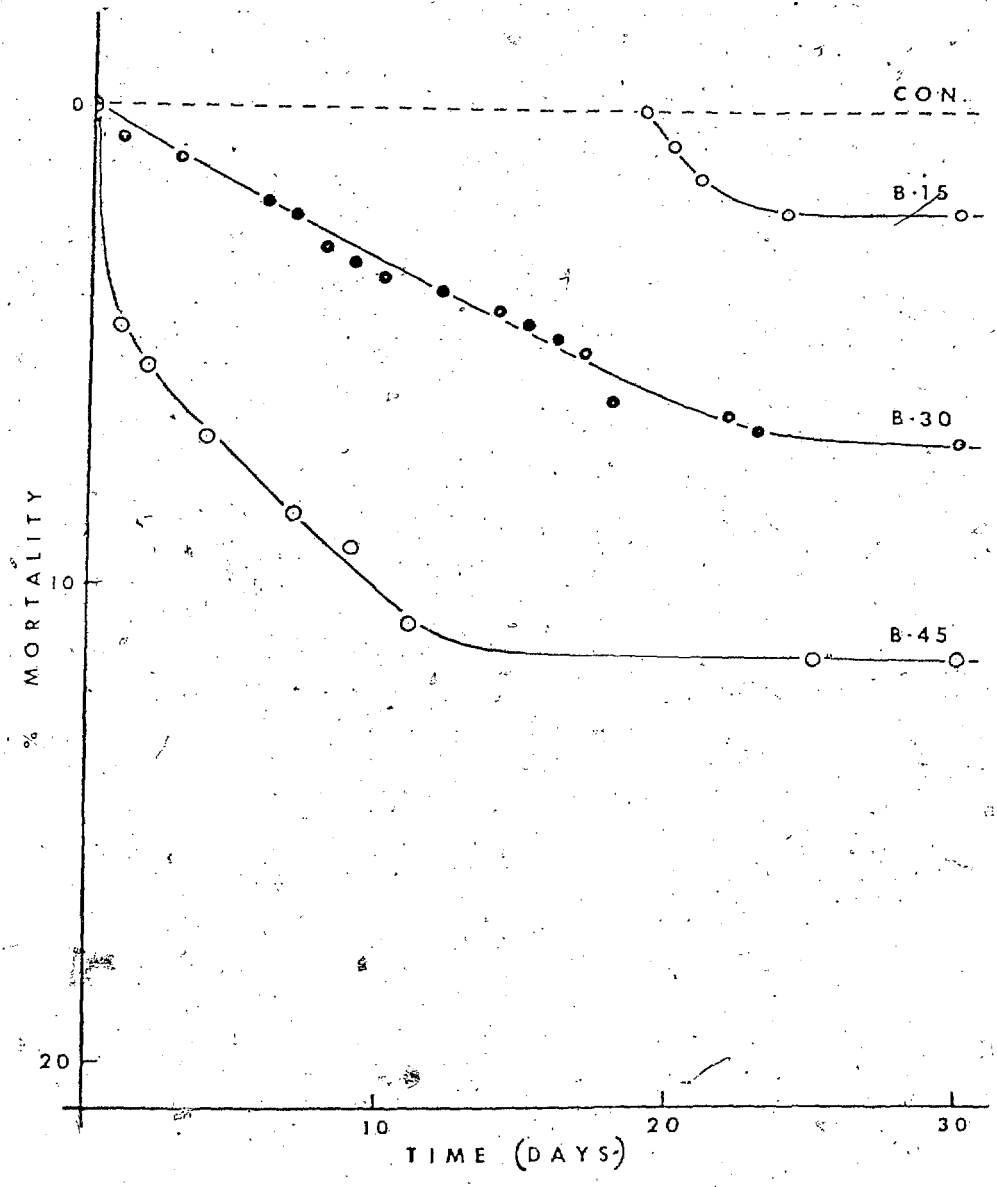

0
40
80
% MORTALITY
CON.
620 R.
800
CUMULATIVE
% MORTALITY
1020
1240
5030
2300
10
20
30
TIME (DAYS)

Figure 14: Percent Mortality Cumulative (30 Days); Thermal Stress (°).


CON.
B·15
B·30
B·45
0
10
20
% MORTALITY
10
20
30
TIME (DAYS)

of mice subjected to fallout stress (Fig. 15).

For all time-mortality analyses the combined run mortality has not been corrected (adjusted) for burn mortality. Such an adjustment was only possible when dealing with total 30 day mortality. The differing number of animals between runs prevents a daily correction for mortality. However, the nature of the curve remains essentially the same whether the adjustment is made or not, since the response of burn alone is linear. The uncorrected data also allows the earlier onset of mortality to be seen when the more severe thermal exposures are combined with fallout.

Supralethal doses are less damaging when delivered at a constant rate than at a fallout rate; after 5030 R of F, all animals are dead by day 6, but after 5000 R of CR, complete lethality is not reached until day 9 (Fig. 16).

With increasing burn there is an increasingly earlier onset of mortality, especially noticeable at higher doses (e.g. 1100 R). Following B.15 + CR treatment a definite synergistic effect is manifest over the entire 30 day period, becoming most definite over the latter half of the time course (Fig. 17). This observation is quite opposed to that made when B.15 + F is compared with F. In that case there is little difference noticeable between the two treatments. However, B.30 + F treatment causes a definite protective effect over the latter half of the time course following a higher early mortality.

Figure 15: Percent Mortality Cumulative (30 Days); Fallout Treatments.

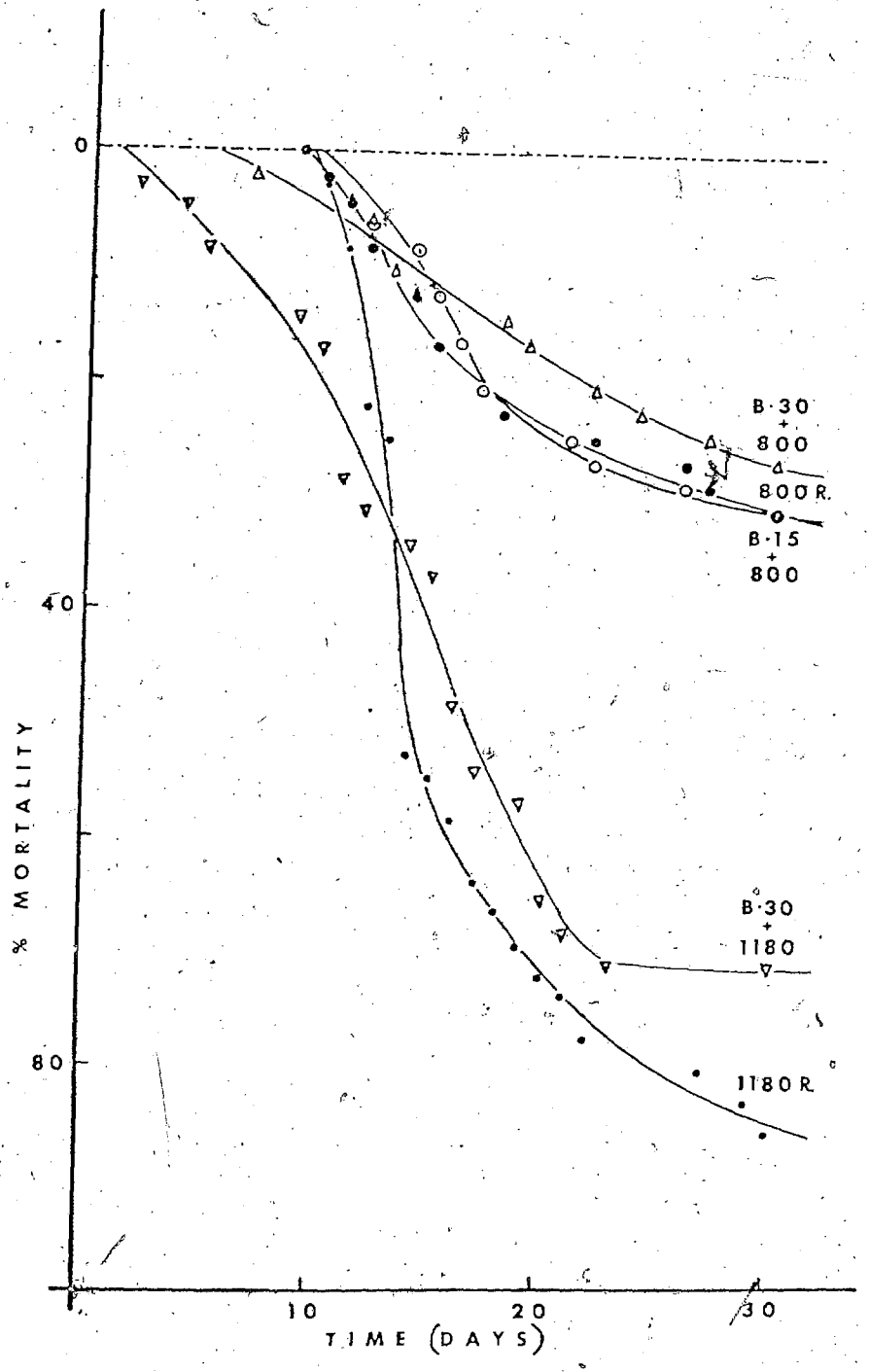

0
40
80
% MORTALITY
10
20
30
TIME (DAYS)
B·30 + 800
800 R.
B·15 + 800
B·30 + 1180
1180 R.

Figure 16: Percent Mortality Cumulative (30 Days); Constant Rate Stress (CR).
All deaths recorded are expressed by the accumulated percent mortality by the day on which the deaths occurred.

The dose represented by each curve is indicated on the graph.

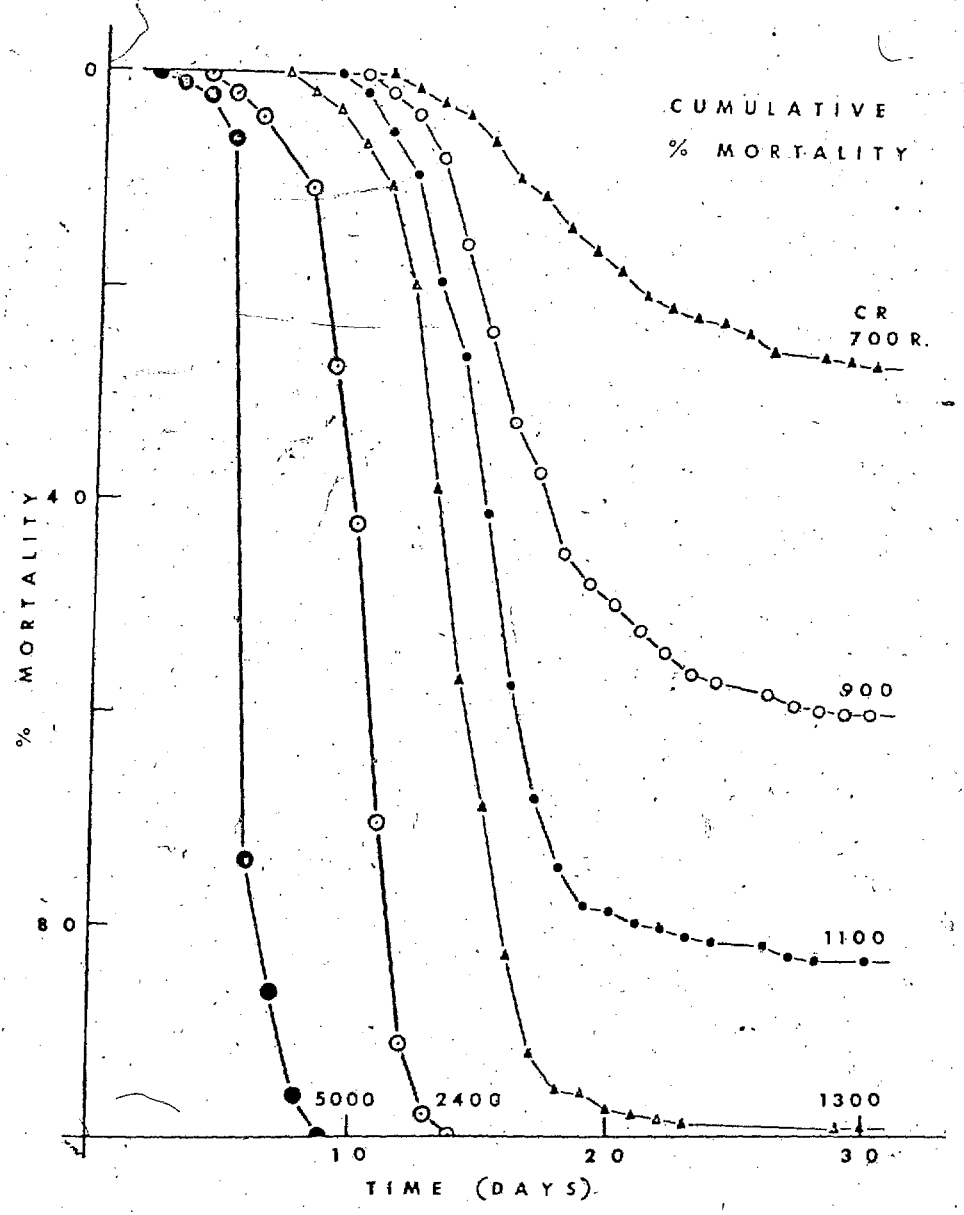
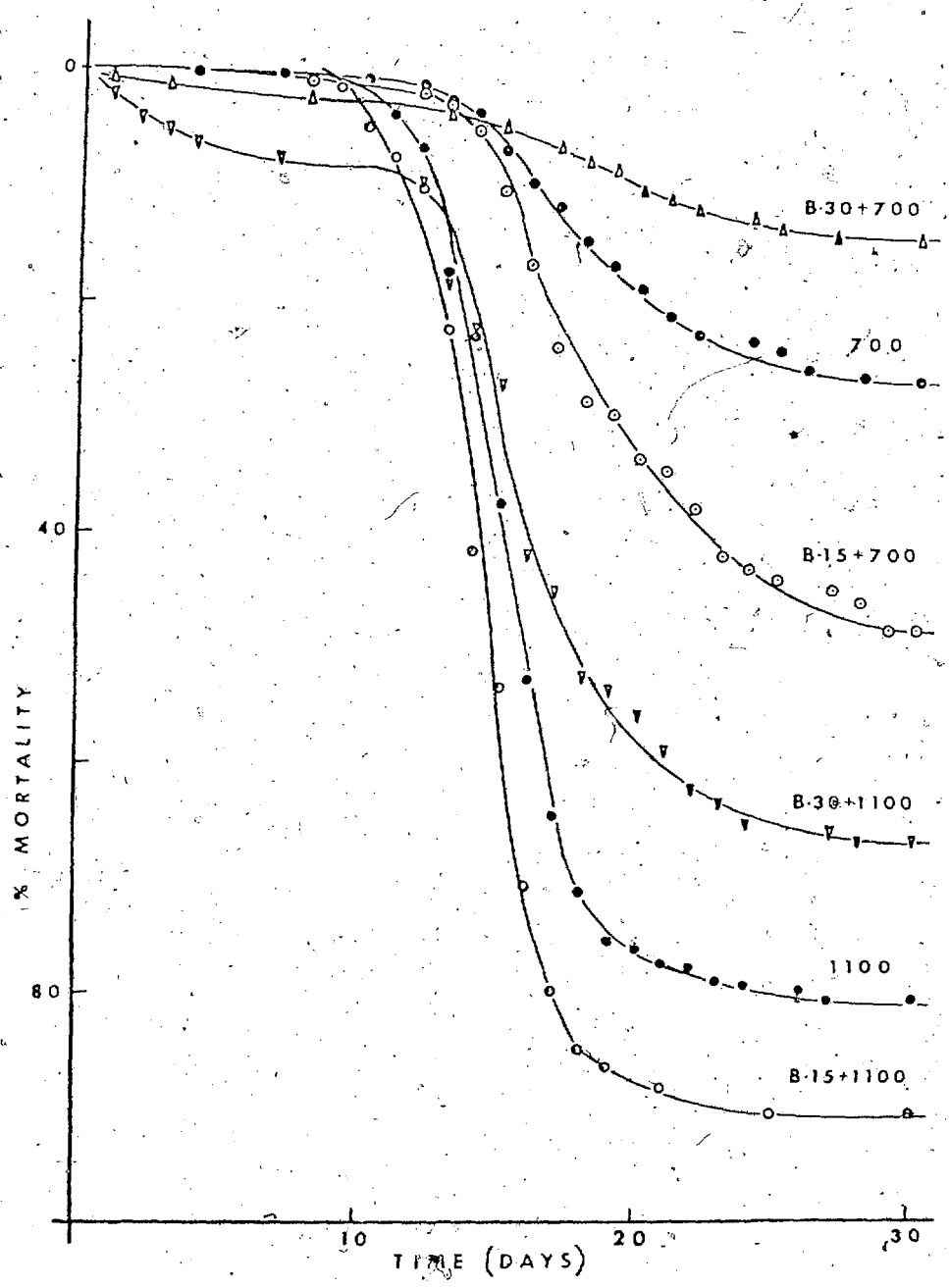

CUMULATIVE
% MORTALITY
CR
700 R.
900
1100
1300
5000
2400
0
40
80
% MORTALITY
10
20
30
TIME (DAYS)

Figure 17: Percent Mortality Cumulative (30 Days); Constant Rate Treatments.

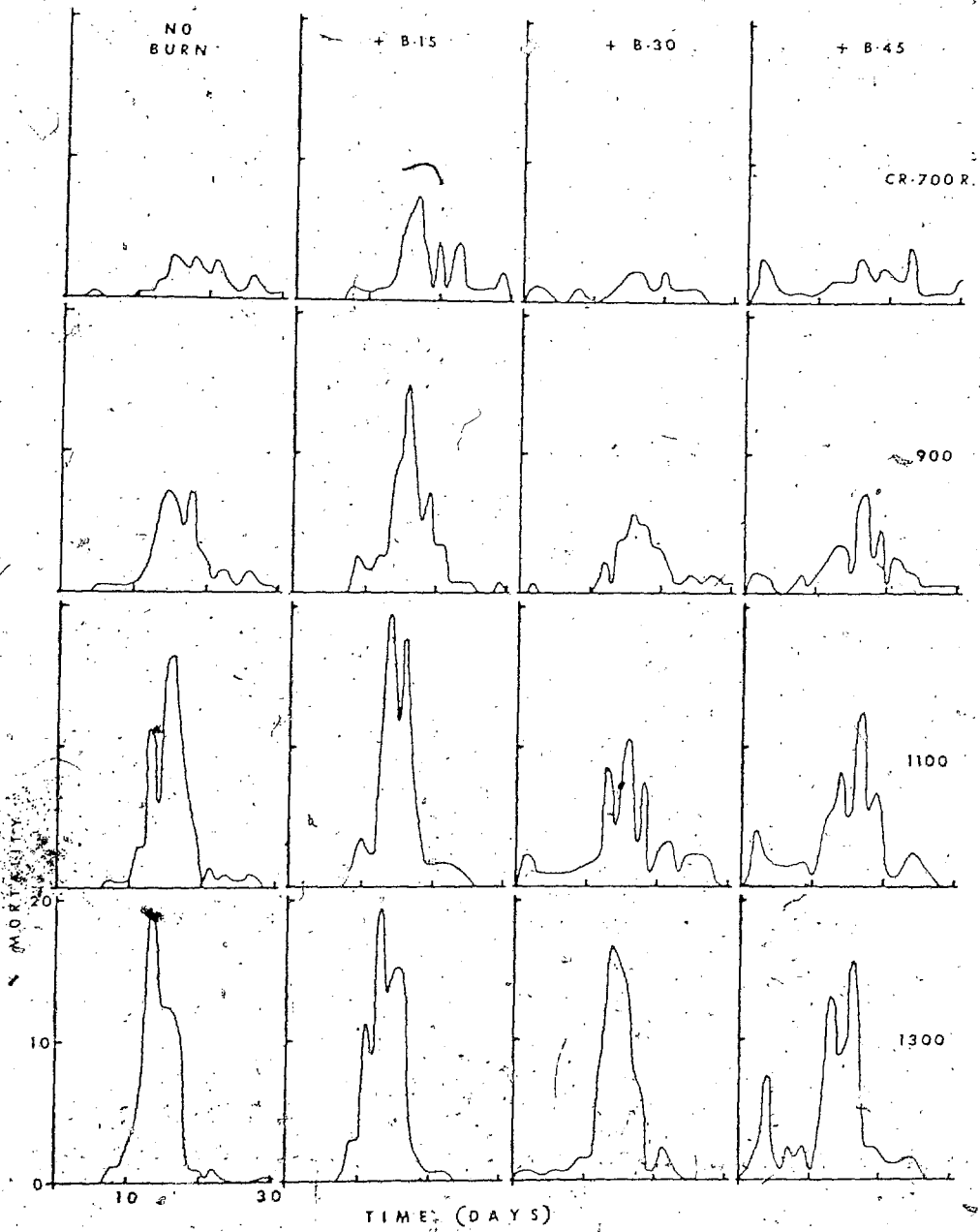

0
40
80
% MORTALITY
10
20
30
TIME (DAYS)
B·30+700
700
B·15+700
B·30+1100
1100
B·15+1100

Death Distribution

Most deaths were observed to occur between days 12 and 20, indicative of hematopoietic failure. The protective versus synergistic effects may be differentiated primarily by the decrease or increase in mortality during this time period. To further investigate and confirm this observation a death distribution table was prepared for each treatment group to study when deaths occurred. The time periods used each correspond to a particular mortality syndrome. Deaths in the 0 - 6 day period are attributable to the gastrointestinal syndrome (or shock), in the 7 - 11 day period to the bacteremic, late gastrointestinal or early bone marrow syndromes, in the 12 - 20 day period to the bone marrow syndrome and in the 21 - 30 day period to the late bone marrow syndrome.

Mortality fluctuates over the 30 day period of observation with the most notable variations occurring in the 12 - 20 day period (Fig. 18). Variation in the height of a mortality peak indicates the severity of the stress on the animals. The location of the peak, the time period in which it occurs, indicates the syndrome to which the animals most probably succumbed. The mortality peak reaches a maximal height following the 15 second burn plus constant rate radiation treatment, and its least following a 30 second burn plus radiation. The 45 second burn plus radiation results in a peak intermediate between the peaks of radiation alone and the 30 second burn plus radiation in the mid-lethal range (900 R to 1100 R). At 700 R the mortality is generally too

Figure 1 : Daily mortality (30 day period); Constant rate Perimex (μ + CR).
The percent mortality is plotted against the time (days) for the various combinations of thermal stress and constant rate radiation. Each column represents a particular thermal exposure and each row represents a particular radiation dosage. Lines have been drawn to give the best fit to the data.


NO BURN
+ B·15
+ B·30
+ B·45
CR·700 R.
900
1100
1300
% MORTALITY
20
10
0
10
30
TIME (DAYS)

low to show much deviation in this type of plot and mortality tends to be rather diffuse over the 30 days.

The 1100 R treatment groups give a very clear picture of the variations in mortality, so these will be discussed in more detail. The 3.15 + 1100 R combined stress results in the highest mortality peak, followed by 1100 R alone, then 3.45 + 1100 R, and finally, 3.30 + 1100 R. With the first two treatments, the mortality peak is actually composed of two peaks between days 12 and 20. With 1100 R alone the second peak is the higher, but with 3.15 + 1100 R the increase in mortality occurs slightly earlier so the first peak becomes the higher. Thus, combining 3.15 with 1100 R seems to increase lethality in the early bone marrow phase.

For 3.30 + 1100 R and 3.45 + 1100 R the mortality peak is actually composed of three peaks in the 12 - 20 day period. With 3.30 + 1100 R the middle peak tends to be the greatest, and this is accentuated with 3.45 + 1100 R. Combining 3.30 or 3.45 with constant rate radiation tends to increase mortality in the mid-bone marrow phase.

Early mortality from days 0 to 6 caused by thermal stress alone becomes more evident as the thermal exposure is increased. There also appears to be an increase in late deaths from days 21 to 30.

Syndrome Mortality; Fallout Radiation

Each time period has a peak in mortality, a maximum mortality, at a particular fallout dose or narrow range of

doses (Table 8). As the dose is increased a peak is attained; then deaths decline. During this decline in one time period deaths are on the increase in the next earlier period. Mice subjected to 670 R of fallout radiation are most likely going to die in the period from days 21 to 30; if subjected to 2300 R they would most likely succumb by day 11, and to 5030 R, by day 5. In Table 8 the shift from later syndromes to earlier ones with increasing dose is easily observed when the doses causing 50% mortality are noted in each time period.

The mortality peaks in the 7 to 11 and 12 to 20 day periods are quite pronounced, while rather diffuse in the 21 to 30 day period (Fig. 19). As the mortality in one period decreases with increasing dose, the mortality in the next earlier period is increasing. The broad peak has a median value in the 21 to 30 day period of about 1000 R, in the 12 to 20 day period of 1200 R, in the 7 to 11 day period of 2300 R, and the 0 to 6 day period of 4000 R. The earliest syndrome period (days 0 - 6) does not have an actual peak, but rather a plateau at 100% lethality. The small peak in the first period at 1460 R is probably not significant as it is only encountered at that one dose and at no others just above or below it.

The decedent survival time decreased as the dosage was increased since more animals were dying earlier. Table 9 records the decedent survival time, the average survival time of decedents, which was calculated by adding together the days

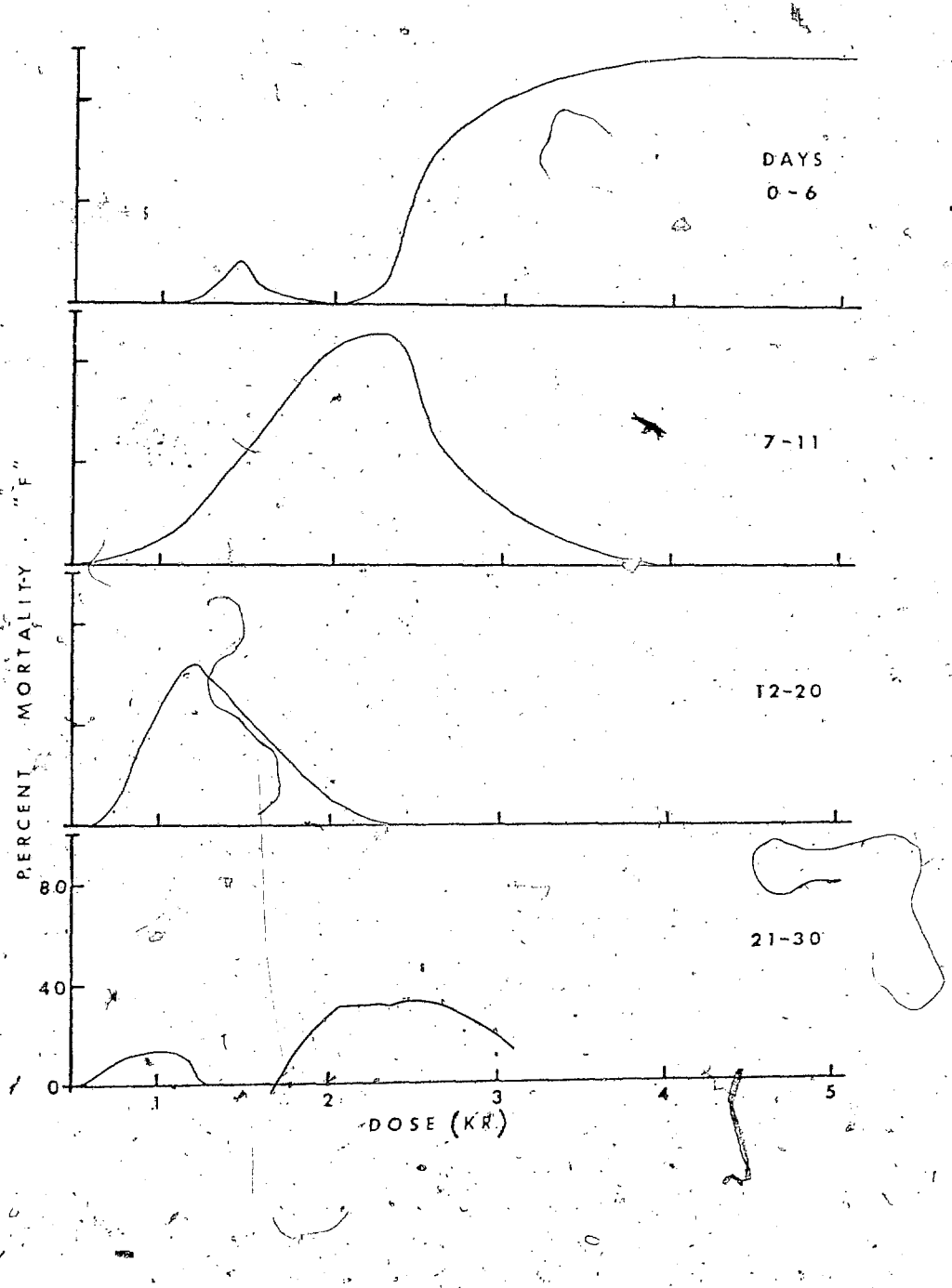
TABLE 8

TIME-MORTALITY ANALYSIS; FALLOUT STRESS

DOSE (R)	PERCENT MORTALITY DAYS 0-6	DAYS 7-11	DAYS 12-20	DAYS 21-30	TOTAL	DECEDENT SURVIVAL TIME (DAYS)*	
620	0	0	4.6	1.5	6.1	22.2	± 1.9
670	0	0	2.8	13.9	16.7	18.5	4.0
702	2.7	5.6	11.1	5.6	25.0	15.9	2.6
900	0	4.2	18.8	8.3	31.3	17.5	1.6
930	0	2.9	34.3	11.4	48.6	17.8	1.1
925	0	2.1	35.5	10.4	50.0	17.6	1.2
955	0	6.3	37.5	12.5	56.3	17.7	1.1
1020	0	8.3	50.0	2.8	61.1	14.8	0.6
1044	0	10.4	56.2	6.3	72.9	15.2	0.8
1070	0	5.6	47.2	13.9	66.7	16.3	1.0
1095	0	22.2	55.5	5.6	83.3	13.3	0.8
1180	0	8.3	63.9	13.9	86.1	16.0	0.9
1240	0	26.7	60.0	3.3	90.0	13.4	0.7
1256	2.0	2.0	62.0	32.0	96.0	13.9	0.5
1460	16.7	50.0	33.3	0	100	10.1	0.5
1550	2.8	50.0	47.2	0	100	10.8	0.4
1700	3.3	63.4	33.3	0	100	10.4	0.4
2300	8.8	91.2	0	0	100	8.3	0.2
2600	50.0	50.0	0	0	100	6.8	0.2
2700	96.7	3.3	0	0	100	4.5	0.1
3030	100	0	0	0	100	4.6	0.1

* The mean survival time of decedents (see p. 56), ± 1 SE

Figure 14: Syndrome Mortality; Fallout Stress.
The percent mortality is plotted against the fallout dose for each time period.


DAYS
0 - 6
7 - 11
12 - 20
21 - 30
PERCENT MORTALITY "F"
80
40
0
1
2
3
4
5
DOSE (KR)

on which each death occurred. For example, if two animals died on day 9, three on day 12 and four on day 15, the DST value would be:

$$\frac{(2 \times 9) + (3 \times 12) + (4 \times 15)}{2 + 3 + 4} = 11.8 \text{ days}$$

When the burn plus fallout radiation mortality results are analysed by time period (Table 9) the mortality rises and probably reaches a peak in each period, thereafter declining. However, the range of doses employed for combined stress treatments did not exceed 1256 R so in most periods the peak was not actually attained. It can be seen that by far the greatest number of deaths always occur in the 12 - 20 day period whether the treatment is fallout radiation alone or burn plus fallout radiation unless supra-lethal doses greatly exceeding 1460 R are employed as in Table 8. Figure 20 graphically illustrates the foregoing points with respect to the trends in mortality. As the degree of burn is increased the proportional amount of mortality in the first 6 days increases.

Most deaths occur in the 12 to 20 day period. Although each regime displays essentially the same dose response in this period there is less mortality following 5.30 + F and 5.45 + F in contrast to 5.15 + F and fallout alone; the curves are shifted to the right by the addition of 2° - 3° burns (5.30 and 5.45), indicating protection. With the 45 second combined treatment some deaths are shifted from the 12 to 20 day period to the earlier 7 to 11 day period.

TABLE 9

TIME-MORTALITY ANALYSIS; BURN PLUS FALLOUT STRESS

BURN (SEC)	DOSE (R)	PERCENT MORTALITY DAYS 0-6	DAYS 7-11	DAYS 12-20	DAYS 21-30	TOTAL	DECEDENT SURVIVAL TIME (DAYS)	
15	0	0	0	0.7	1.4	2.1	23.7	± 1.2
15	800	0	0	20.9	10.4	31.3	17.7	1.4
15	955	0	6.3	39.5	6.3	52.1	15.1	0.9
15	1044	0	4.0	50.0	10.0	64.0	16.4	0.9
15	1256	2.2	19.6	63.0	6.5	91.3	13.8	0.6
30	0	1.9	1.6	2.6	0.9	7.0	11.3	1.4
30	475	0	0	2.1	0	2.1	18.0	-- **
30	800	0	4.2	12.5	10.4	27.1	17.8	1.9
30	925	0	4.2	10.4	16.7	31.3	17.5	1.4
30	955	0	0	24.0	10.0	34.0	18.7	1.1
30	1044	2.1	10.4	41.7	8.3	62.5	15.6	1.0
30	1070	0	13.9	50.0	11.1	75.0	15.3	0.8
30	1190	8.6	20.0	37.1	5.7	71.4	13.8	1.1
30	1256	2.1	16.7	64.5	4.2	87.5	13.8	0.6
45	0	6.9	3.8	0	0.8	11.5	5.7	1.7
45	800	4.5	0	13.7	4.5	22.7	17.0	3.8
45	955	0	4.1	16.7	0	20.8	14.9	1.1
45	1044	8.3	25.0	37.5	0	70.8	11.6	0.9
45	1256	12.5	33.3	50.0	0	95.8	11.9	0.8

± 1 SE

** only one death

Figure 20: Syndrome Mortality; Combined Stress (Burn + Fallout). The percent mortality is plotted against the Fallout dose. Each column represents a particular thermal exposure and each row represents the particular time period being examined.

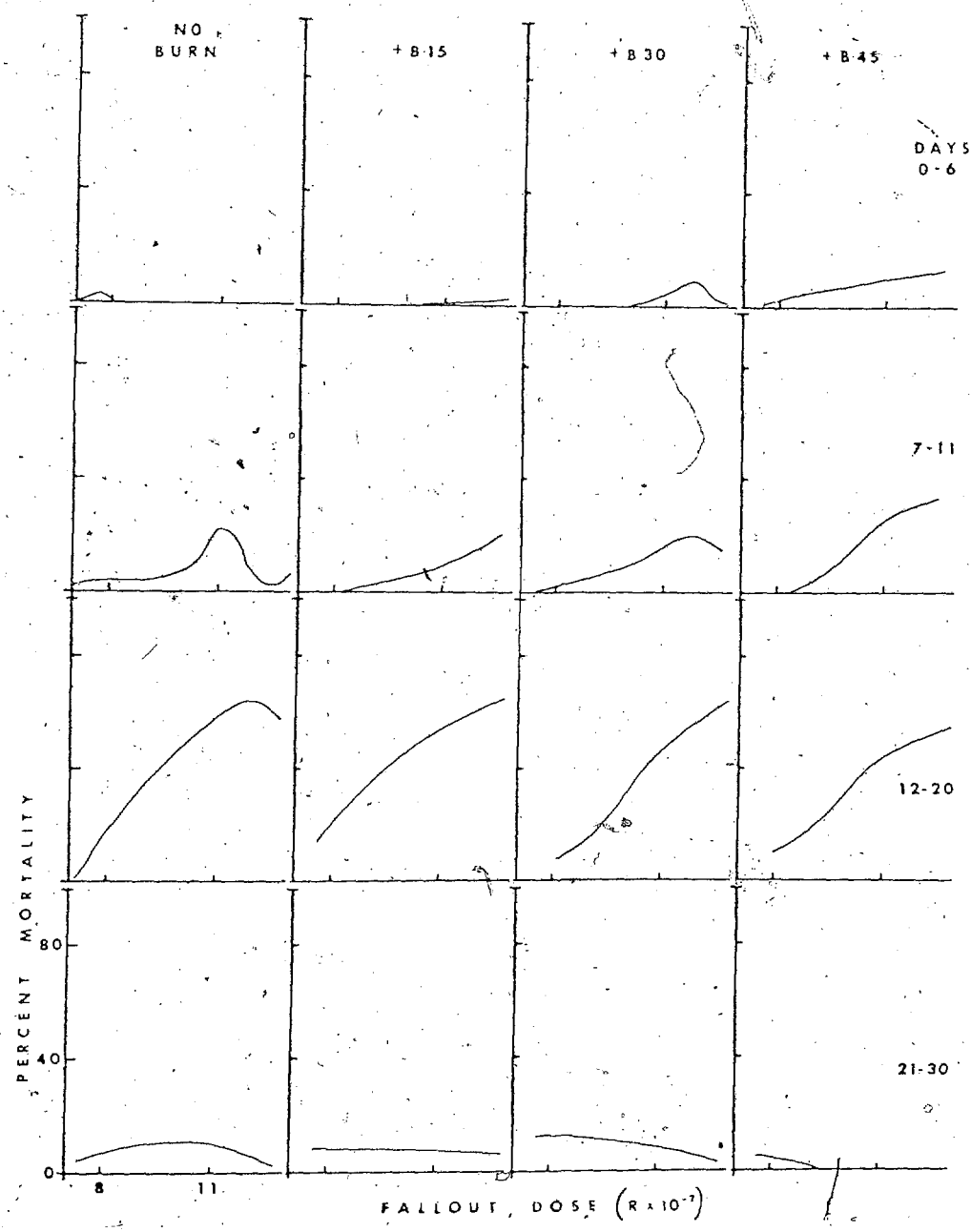

NO BURN
+ B 15
+ B 30
+ B 45
DAYS 0 - 6
7 - 11
12 - 20
21 - 30
PERCENT MORTALITY
80
40
0
8
11
FALLOUT, DOSE (R x 10⁻²)

Table 9 records the decedent survival times for the burn plus fallout regimes. These appear about the same as for fallout alone until the burn stress becomes severe enough to cause a greater early mortality from days 0 to 6.

Syndrome Mortality; Constant Rate Radiation

The mortality from constant rate radiation peaks at a single dose in each time period (Table 10). For supralethal doses radiation delivered at a constant dose rate is less damaging than at a fallout rate (Table 8). After 5000 R of constant rate radiation 74.1% of the deaths occur by day 6, but after 5030 R of fallout radiation 100% of the animals are dead by day 6. This is supported by the decedent survival time of 5.4 days for 5000 R and 4.6 days for 5030 R.

The mortality from constant rate radiation is displayed graphically for each time period in Figure 21. Mortality occurs earlier with increasing dose and the peak of mortality occurs in an earlier time period at higher doses. No deaths are observed in the 0 to 6 day period until supralethal doses are delivered in excess of 2000 R. Even at 5000 R, the highest dose applied, deaths were not limited to the first 6 days as they were with 5030 R under fallout.

When the fallout and constant rate mortality results for each time period are compared (Fig. 22), there is little deviation during the 21 to 30 day period, but a great difference during the three preceding periods. In the 12 to 20 day period the constant rate dose response curve shows a higher mortality

TABLE 10

TIME-MORTALITY ANALYSIS; CONSTANT RATE STRESS

DOSE (R)	PERCENT MORTALITY DAYS 0-6	DAYS 7-11	DAYS 12-20	DAYS 21-30	TOTAL	DECEDENT SURVIVAL TIME (DAYS)*
700	0.4	0.4	17.6	8.2	26.6	18.5 ± 0.6
900	0.5	1.9	45.7	7.7	55.8	16.7 0.4
1100	0	3.8	72.3	4.7	80.8	15.9 0.2
1300	0	4.1	85.6	3.1	92.8	15.2 0.3
1800	0	33.3	66.7	0	100	12.4 0.3
2400	4.2	66.0	29.8	0	100	10.5 0.3
2500	10.4	70.8	18.8	0	100	9.8 0.3
3000	14.6	83.3	2.1	0	100	8.2 0.2
3450	33.3	66.7	0	0	100	7.4 0.2
4000	66.7	33.3	0	0	100	6.5 0.1
5000	74.1	25.9	0	0	100	6.3 0.1

* ± 1 SE

Figure 21: Syndrome Mortality; Constant Rate Stress. The percent mortality is plotted against the constant rate dose for each time period.

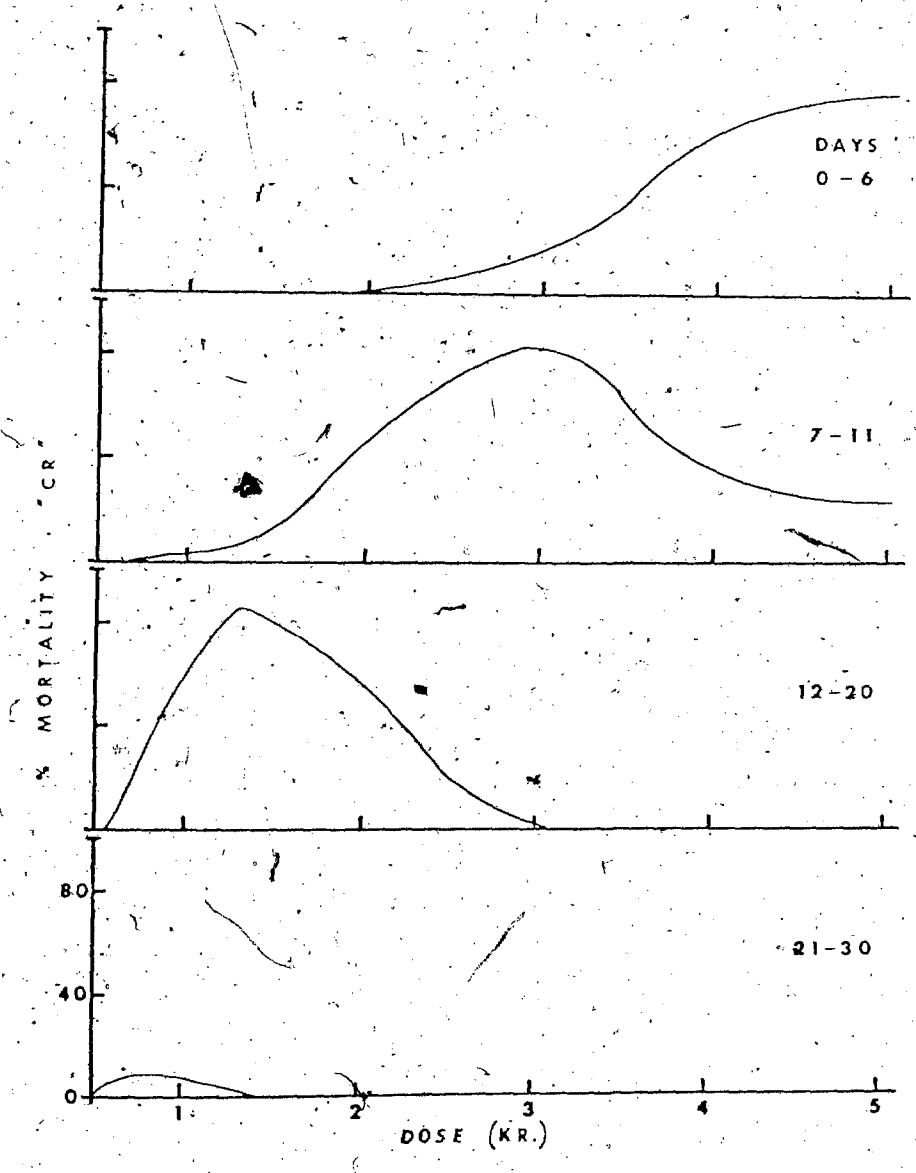
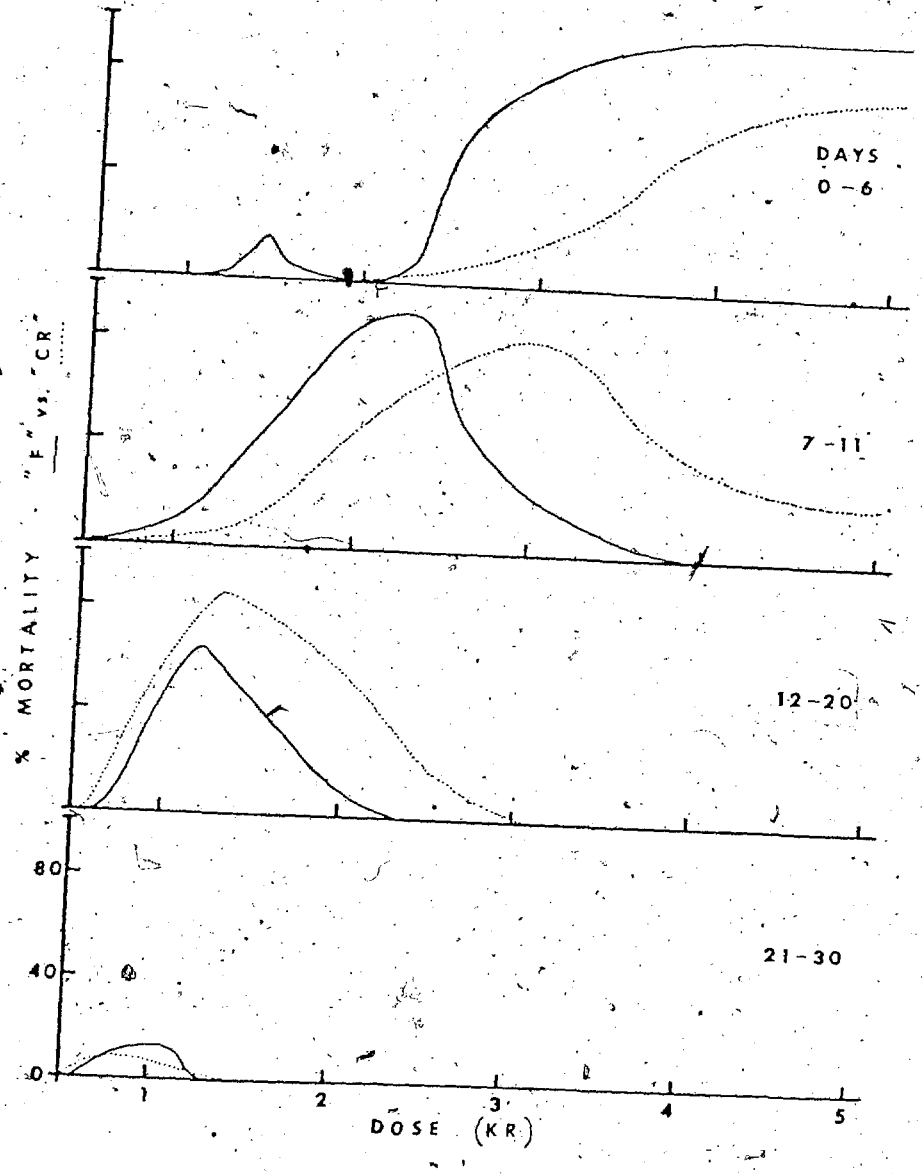

DAYS
0 - 6
7 - 11
12 - 20
21 - 30
% MORTALITY "CR"
80
40
0
1
2
3
4
5
DOSE (KR.)

Figure 22: Syndrome Mortality; Fallout Versus Constant Rate Stress.

Code: solid line, fallout
dotted line, constant rate


DAYS
0 – 6
7 – 11
12 – 20
21 – 30
% MORTALITY "F" vs. "CR"
80
40
0
1
2
3
4
5
DOSE (KR)

over a broader range of doses than does the fallout curve. In the 0 to 6 and 7 to 11 day periods the dose response curve for constant rate doses is shifted to the right of the fallout curve. This delayed response is quite noticeable, particularly in the 7 to 11 day period. The difference between fallout and constant rate becomes greater as higher supralethal doses are employed. The constant rate curve seems to plateau at a different level of mortality than the F curve.

The decedent survival times of Tables 9 and 10, for fallout and constant rate stress respectively, reflect the preceding observation (Fig. 23), where there is a consistent deviation in sensitivity between animals subjected to fallout and those subjected to constant rate stress. Up to about 1000 R the constant rate stress is apparently about the same as fallout stress in terms of lethality, but above 1000 R the constant rate stress produces less lethality.

The major mortality with burn plus constant rate radiation occurs, as with the radiation alone, between days 12 and 20 (Table 11). In this time period a prior 15 second thermal exposure markedly increases the mortality caused by the radiation alone (Table 10), while both 30 second and 45 second exposures markedly decrease the mortality. Since all these variations occur primarily in the one time period, it would be expected that the decedent survival times would not differ to any great extent between the combined treatments and the radiation alone; this is indeed the case. There is a slight tendency to earlier

Figure 27: Decedent Survival Time; Fallout Versus Constant Rate Stress.
The mean survival time of decedents in days is plotted against the dose in HR (1000 R).

Code: cloned circles, F
open circles, CP

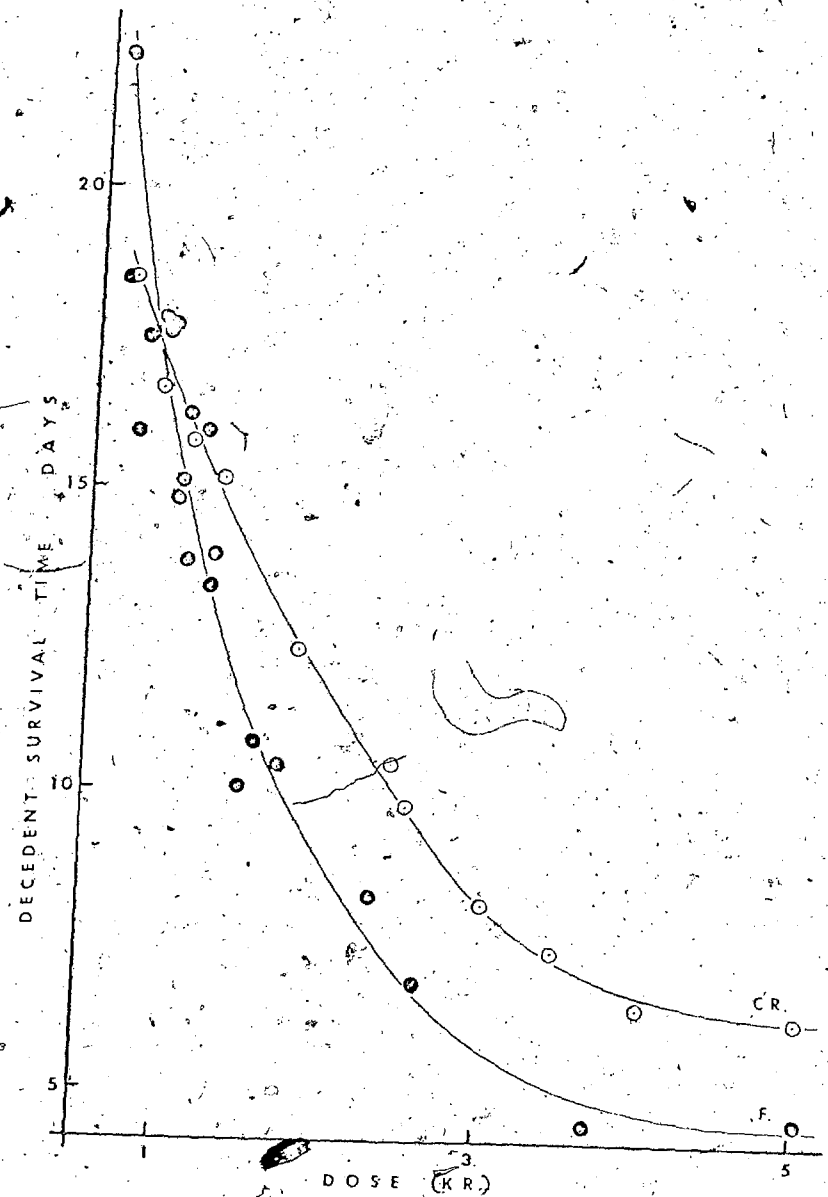

DECEDENT SURVIVAL TIME DAYS
20
15
10
5
1
3
5
DOSE (KR.)
C.R.
F.

TABLE 11

TIME-MORTALITY ANALYSIS; BURN PLUS CONSTANT RATE STRESS.

BURN (SEC)	DOSE (B)	PERCENT MORTALITY DAYS 0-6	DAYS 7-11	DAYS 12-20	DAYS 21-30	TOTAL	DECEDENT SURVIVAL TIME (DAYS)	±
15	0	0	0	0.7	1.4	2.1	21.7	1.2
15	700	0	1.1	32.6	14.7	48.4	18.8	0.7
15	900	0	6.2	64.6	8.0	78.8	16.4	0.4
15	1100	0	7.6	80.5	2.6	90.7	14.9	0.2
15	1300	0	4.6	90.9	0	95.5	15.2	0.4
30	0	1.9	1.6	2.6	0.9	7.0	11.3	1.4
30	700	1.7	0.8	7.6	4.2	14.3	16.8	1.8
30	900	0.8	0.8	33.3	9.8	44.7	17.8	0.6
30	1100	6.4	1.1	48.4	12.9	68.8	15.7	0.7
30	1300	4.2	2.7	77.8	4.2	88.9	14.5	0.5
45	0	6.9	3.8	0	0.8	11.5	5.7	1.7
45	700	7.3	1.6	14.6	10.6	34.1	15.6	1.3
45	900	3.4	4.7	30.4	9.5	48.0	16.3	0.7
45	1100	9.2	4.2	58.3	6.6	78.3	14.6	0.6
45	1300	23.6	5.6	59.7	1.4	90.3	11.8	0.7

± 1 SE

values with B.30 + CR and with B.45 + CR. This is especially noticeable because the mortality in the 0 to 6 day period is rising as the thermal exposure is increased. The B.45 + 1256 R (fallout) shows this tendency also, but not to the extent shown by the B.45 + 1300 R stress (constant rate).

The results of the death distribution by syndrome period for constant rate radiation and the various thermal exposures (Tables 10, 11) are displayed graphically in Figure 24. In the 12 to 20 day time period there appears to be a shift in the curve that reflects the change in total mortality. The curve is higher in the B.15 + CR group indicating increased lethality and lower in the B.30 + CR and B.45 + CR groups indicating reduced lethality from groups given constant rate radiation alone. With B.45 + CR the mortality seems to plateau at a lower dose of radiation and at a lower level of mortality. This is due in part to the increased mortality in the 0 - 6 day period; the animals normally succumbing in the 12 - 20 day period are now dying sooner. The other time periods (7 to 11 and 21 to 30) show little deviation from one treatment to another.

Figure 25 provides a time period comparison of the mortality from constant rate and fallout radiation for various thermal exposures. Fallout combinations generally exhibit greater mortality than constant rate combinations in the 7 to 11 day period. In the 12 to 20 day period the mortality curves are almost identical in character except that the constant rate

Figure 24: Syndrome Mortality; Combined Stress (Burn + Constant Rate).
The percent mortality is plotted against the constant rate dose. Each column represents a particular thermal exposure and each row represents a particular time period.

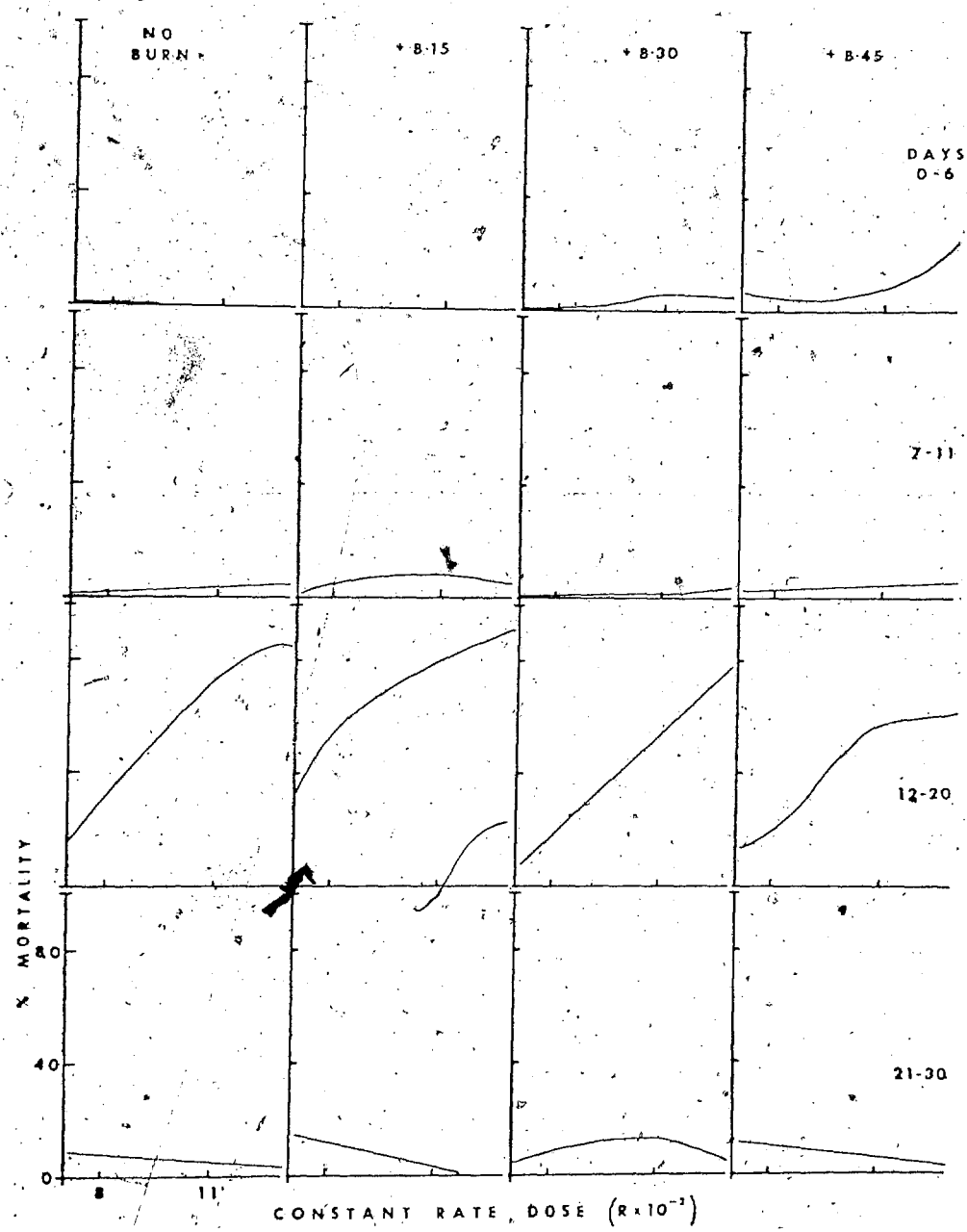
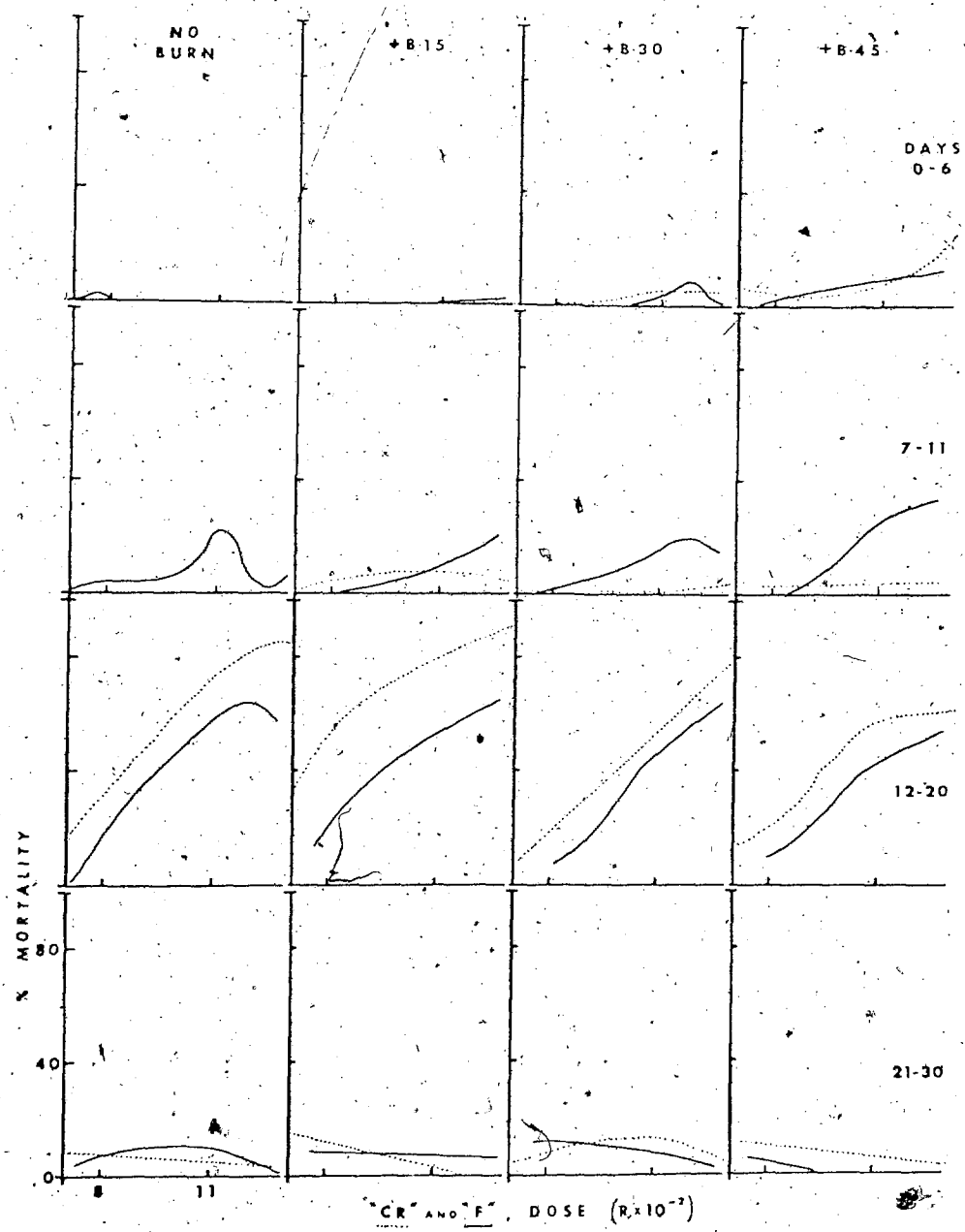

NO BURN
+ 8·15
+ 8·30
+ 8·45
DAYS 0-6
7-11
12-20
21-30
% MORTALITY
80
40
0
8
11
CONSTANT RATE DOSE (R×10⁻²)

Figure 25: Syndrome Mortality; Fallout versus Constant Rate Combined Stress.

Code: solid line, fallout radiation
dotted line, constant rate radiation


NO BURN
+B·15
+B·30
+B·45
DAYS 0-6
7-11
12-20
21-30
% MORTALITY
80
40
0
8
11
"CR" AND "F" DOSE (R x 10⁻²)

treatment curves are consistently higher. That is, a constant rate dose causes a higher mortality than the same total dose of fallout.

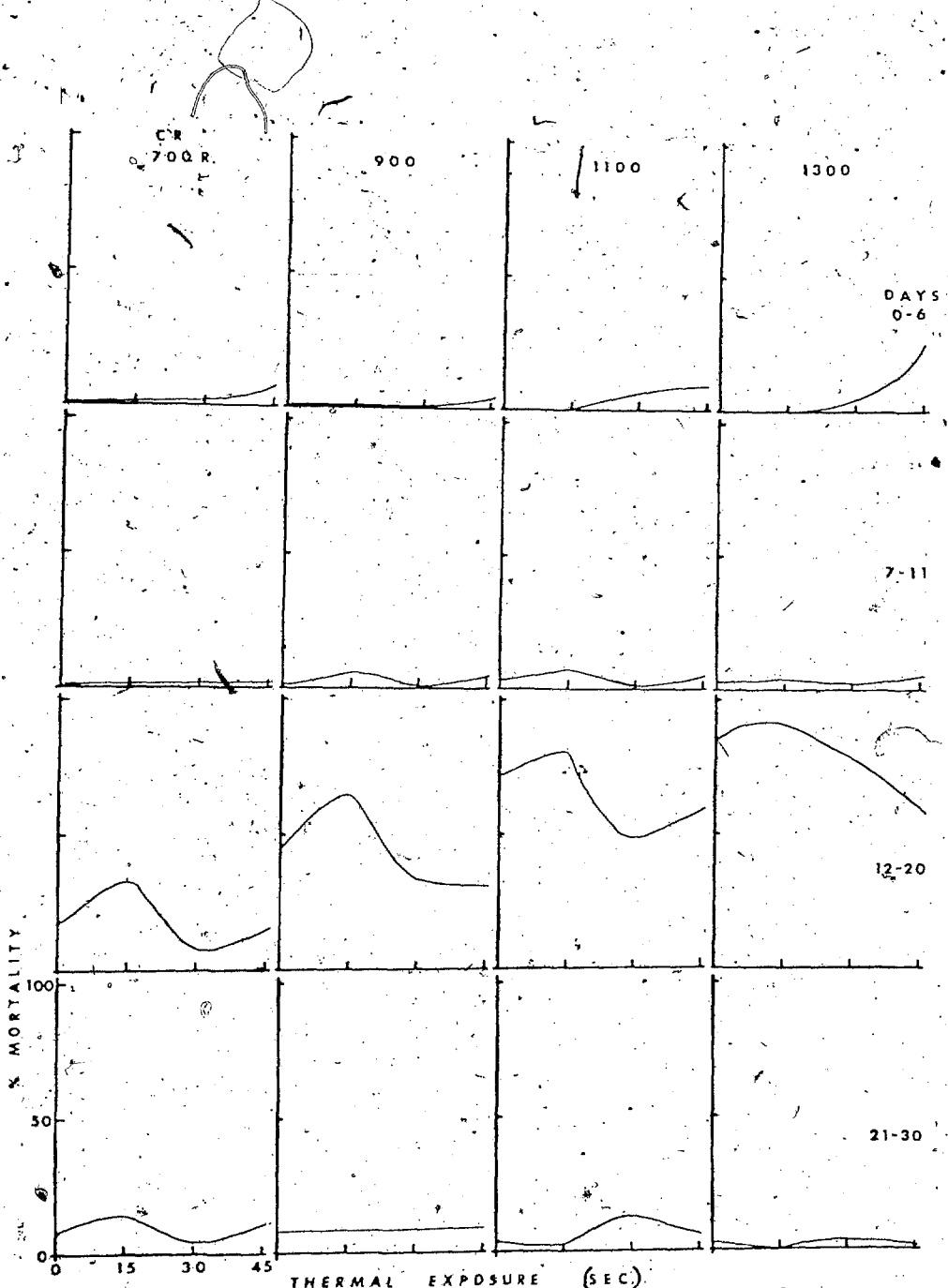
A different way of plotting the death distribution data is shown in Figure 26. The B.15 + CR treatment causes a synergistic (more than additive) effect in the 12 to 20 day period, and the B.30 + CR and B.45 + CR treatments cause a protective effect. Mortality undergoes the most change during this time period as the CR dose is increased, the mortality becoming steadily greater but the shape of the curve remaining essentially the same. The slight deviation in shape with the B.45 + 1300 R treatment has been noted in previous graphs and tables and to some extent balanced by the increased mortality in the 0 to 6 day period.

The mortality in the death distribution analyses could not be adjusted for the burn mortality as with the total mortality for probit analysis because of the variable number of animals in each treatment group. Thus the protective effects of B.30 + CR and B.45 + CR are not exhibited to their fullest extent; they are actually more pronounced than as shown in Figure 26.

BURN-IRRADIATION INTERVAL

When the fallout experiments were performed, the interval between the thermal stress and the onset of fallout radiation was set at about 3 - 4 hours, the time required for the animals

Figure 26: Thermal Stress Analysis; Burn + Constant Rate. The percent mortality is plotted against the thermal exposure. Each column represents a particular dose of constant rate radiation and each row represents a particular time period.


CR
700 R.
900
1100
1300
DAYS
0-6
7-11
12-20
21-30
% MORTALITY
100
50
0
0
15
30
45
THERMAL EXPOSURE (SEC.)

to fully recover from deep anaesthesia. However; a definite time of 3.5 hours was adhered to for the constant rate experiments. Following the preliminary fallout experiments a study was made of the effect on mortality of various time intervals and various constant dose rates (acute to chronic). This study was designed to elucidate any variation in mortality which might result from deviations in the time of onset of irradiation post thermal stress. The results for a 30 second burn combined with three dose rates are recorded in Table #2. The most pronounced effect obtained by varying the time interval between the two stresses is observed with a 900 R dose delivered over 96 hours at 0.16 R per minute (chronic irradiation). Figure 27 graphically displays the results of the study.

Following 900 R of CR irradiation, 56% of the animals succumb in 30 days. When a thermal burn is applied one hour prior to the onset of the radiation 54% mortality is the result. A definite downward trend becomes evident as the interval between burn and onset of irradiation is increased, the minimum mortality of 12.5% being achieved with a 3.5 hour interval. As the interval was further increased the mortality increased reaching a plateau at about 20% with a 4 - 5 hour interval. The brief rise in mortality with a 6 hour interval may not be significant since it was observed in only one run; however that aspect is not of immediate concern to this project as the 3.5 hour interval was always employed. If animals are allowed a 3.5 hour interval between a 30 second thermal stress and

TABLE 12

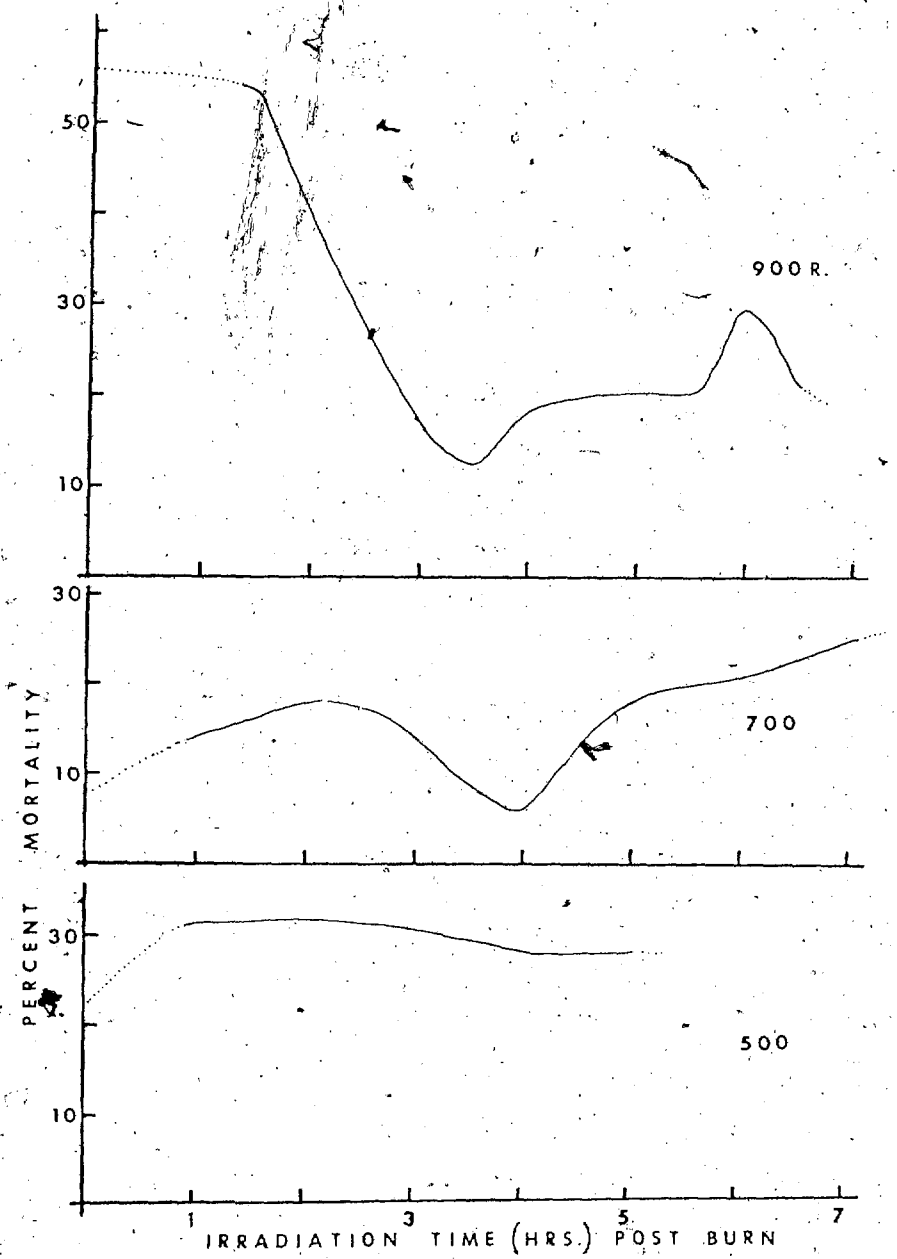
BURN DELAY MORTALITY STUDY FOR THREE DOSE-RATES; 30 DAY MORTALITY

INTERVAL BETWEEN THERMAL EXPOSURE AND IRRADIATION (HOURS)	RADIATION STRESS					
	900 R (96 HOURS) 0.16 R/MIN.		700 R (20 HOURS) 0.58 R/MIN.		500 R (0.5 HOUR) 16.7 R/MIN.	
	ANIMALS USED	PERCENT MORTALITY	ANIMALS USED	PERCENT MORTALITY	ANIMALS USED	PERCENT MORTALITY
0	208	55.8	106	7.5	126	22.2
-1	--	----	49	14.3	51	31.4
-1½	26	53.8	--	----	--	----
-2	50	38.0	50	18.0	51	31.4
-2½	24	29.2	--	----	--	----
-3	89	16.9	50	14.0	52	30.8
-3½	24	12.5	--	----	--	----
-4	87	18.4	50	6.0	50	28.0
-5	87	20.7	50	18.0	50	28.0
-5½	39	20.5	--	----	--	----
-6	50	30.0	24	20.8	--	----
-6½	37	21.6	--	----	--	----
-7	--	----	24	25.0	--	----

--- no experiments performed

Figure 27: Burn-Irradiation Intervals.
Mortality over a 30 day period for various time intervals between a 30 sec. thermal stress and the onset of gamma irradiation is shown for three doses at three different dose rates (Table 12).

Code: 900 R represents 0.16 R/min.
700 R represents 0.58 R/min.
500 R represents 16.7 R/min.


50
30
10
900 R.
30
10
700
PERCENT MORTALITY
30
10
500
1
3
5
7
IRRADIATION TIME (HRS.) POST BURN

radiation, a maximum amount of protection is afforded them. In contrast, a different effect is elicited from 15 second burn combinations; a synergistic effect is exerted on mortality.

The effect of altering the interval between a 30 second thermal stress and 700 R delivered over 20 hours at 0.58 R per minute is not as marked as that with the 900 R dose. The maximum protective effect is now obtained with a 4 hour delay interval. There appears to be no resulting protective effect from combining burn with 500 R delivered over 0.5 hour at 16.7 R per minute (acute irradiation). An acute dose of 900 R could not be used for comparison with chronic 900 R because mortality would have been 100%.

The protective effect exerted by a 30 second thermal stress is dependent on both the time before irradiation and the radiation dose rate, being most evident with an interval of 3.5 hours and a chronic dose rate of 0.16 R/minute.

ENDOGENOUS SPLEEN COLONIES

From the mortality data it can be seen that most of the deaths occur in the 14 - 20 day period over the range of doses employed (except for the high supra-lethal doses above 2000 R). Also, the synergistic and protective effects of the total mortality data are reflected in the mortality of this time period. Lethality during this time period is known to be caused by hematopoietic failure, the bone marrow syndrome, so the next step was to investigate damage and recovery in the hematopoietic system. An endogenous spleen colony assay was performed to study the effects of radiation and of radiation

mediated by a prior thermal stress upon the hematopoietic system.

The spleen colony counts (number of colonies per spleen) were used to calculate the geometric median number of colonies for each treatment group (Table 13) after the method of Smith et al (1966). With increasing dose there is a decreasing number of colonies per spleen. Also, as the thermal exposure is increased, the number of colonies per spleen increases until a 45 second exposure is applied after which the values begin to decrease. The 45 second burn plus radiation groups fall about midway between the 15 second and 30 second burns combined with radiation.

The relationships between the various treatment groups is displayed graphically in Figure 28. The 30 second burn combined with radiation is definitely protective based on ± 1 SE, but the 15 second burn does not seem to vary significantly from the colony number of mice treated with radiation alone.

The D_0 values for the treatment groups (Table 13) are identical. The regressions fitted to the points in Figure 28 are linear and the values for their slopes are identical; they are parallel relationships.

SPLENIC DRY WEIGHTS -

The splenic dry weight was employed as another parameter to assess the effects of various regimes of radiation on hemopoiesis. When the colonies had been counted, the spleens

TABLE 13

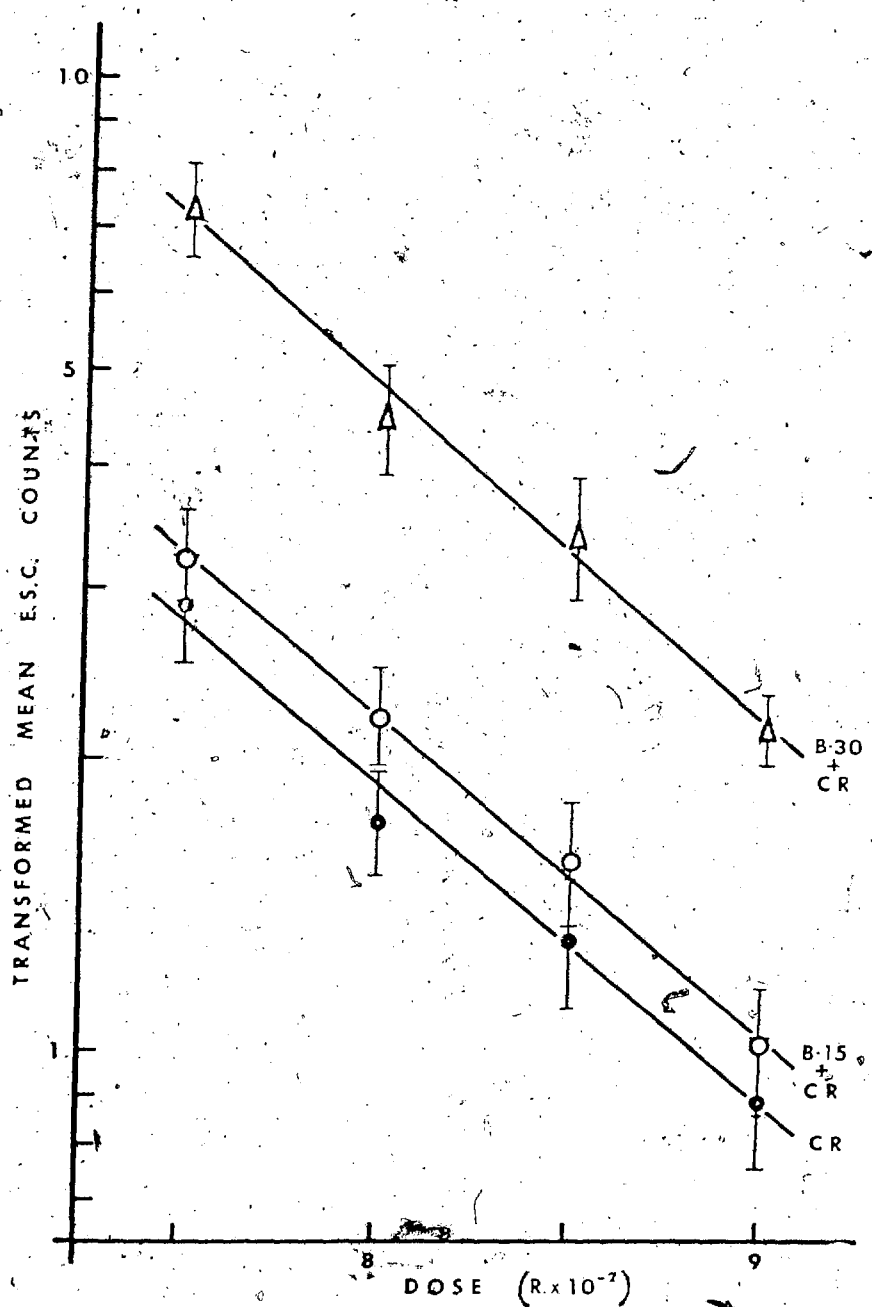
ENDOGENOUS SPLEEN COLONY ASSAY
SHOWING MEDIAN NUMBER OF COLONIES (DAY 12) UNDER CR REGIMES

BURN (SEC)	DOSE (R)[a]	SPLEENS	Mg*		D_0
0	750	124	2.88	± 0.36	
0	800	144	1.73	0.21	131
0	850	99	1.31	0.20	
0	900	100	0.89	0.14	
15	750	123	3.21	0.38	
15	800	142	2.23	0.25	131
15	850	98	1.58	0.23	
15	900	96	1.01	0.15	
30	750	115	7.31	0.81	
30	800	127	4.51	0.57	127
30	850	96	3.44	0.49	
30	900	93	2.18	0.19	
45	750	34	4.79	1.19	
45	800	41	3.06	0.65	133
45	850	42	2.21	0.48	
45	900	100	1.53	0.30	

Mg = geometric median number of colonies

± 1 SE

Figure 28: Endogenous Spleen Colony Survival Curve (Day 12); Constant Rate Treatments.
Log (Mg), where Mg is the geometric median number of colonies, is plotted as a function of dose. Survival uncertainties are shown by ± 1 SE. Codes are as indicated on the graph.


10
5
1
TRANSFORMED MEAN E.S.C. COUNTS
B·30
+
CR
B·15
+
CR
CR
8
9
DOSE (R x 10⁻⁷)

were dried at 100° C for 24 hours and weighed. The mean dry weight in milligrams of the spleens for each stress is recorded in Table 14.

The trends of mortality appear accurately reflected by the changes in the splenic dry weights. The spleens of animals treated with a prior 30 second burn have a greater mean weight than those given radiation alone. Following a 15 second exposure and radiation the weight is lower than after radiation alone. The weights are higher with a 45 second burn than with the 30 second burn combined with constant rate. The opposite relationship is present in the ESC assay. The dry weight is seen to increase with severe burns (no radiation). No colonies were present on the spleens of mice receiving burn alone or from control mice. The radiation was observed to decrease the splenic dry weight to about 30% of control.

The above observations of splenic dry weight are illustrated by a histogram (Figure 29). The trends in dry weight for each stress treatment are consistent for each dose of radiation, and reflect the trends observed in the mortality results; a 15 second thermal exposure will increase the mortality of irradiated animals, but a 30 second thermal exposure will decrease the mortality.

The control spleens were removed from animals that had received no stress other than normal handling. The spleens were weighed by cage lot so the standard error is based on this unit. The control and thermally stressed animal's spleens were weighed as unit groups so no error was calculable.

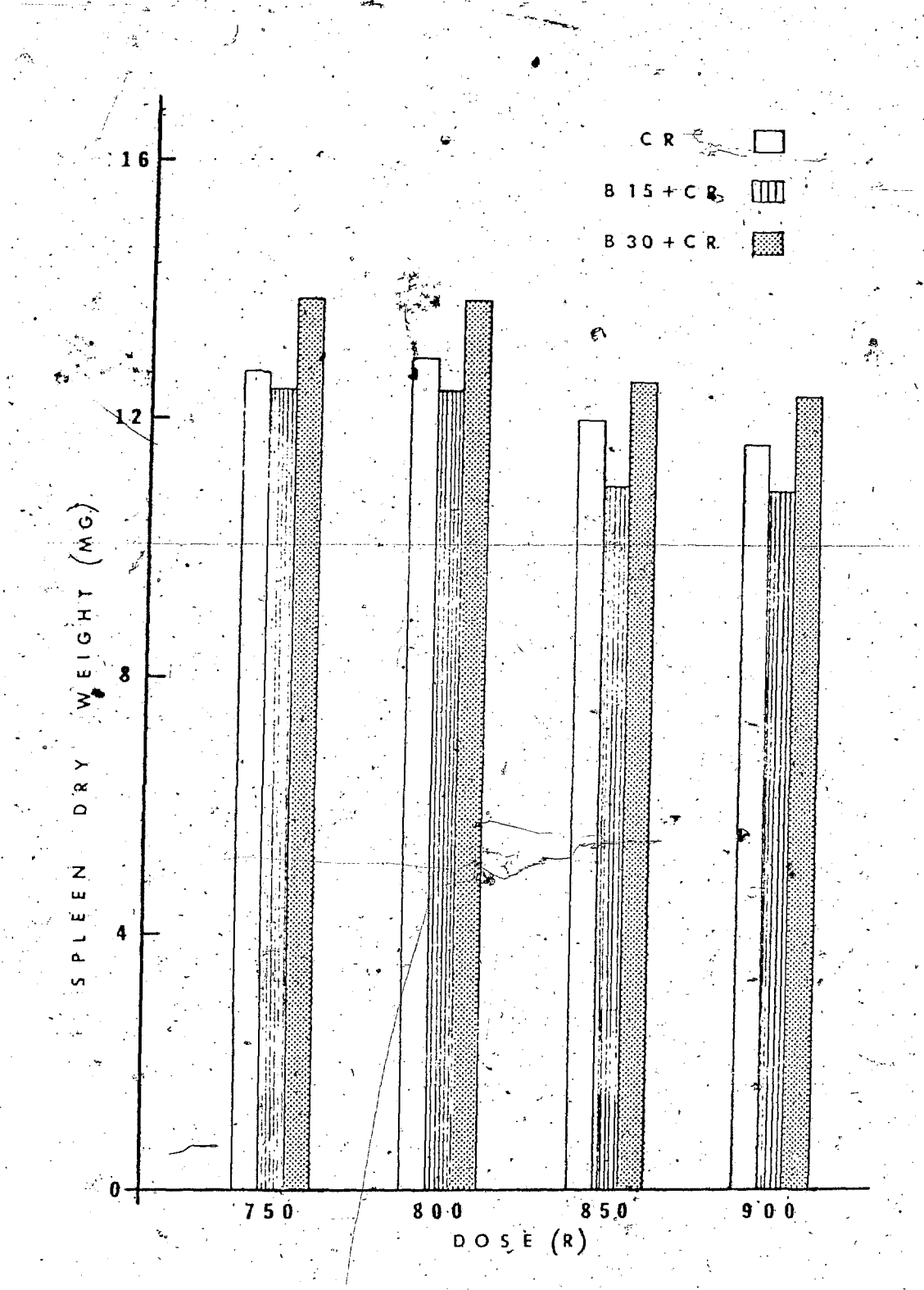
TABLE 14

SPLENIC DRY WEIGHT; (DAY 12) UNDER CONSTANT RATE REGIMES

BURN (SEC)	DOSE (R)	SPLEENS	MEAN DRY WEIGHT (mgm.)*	
0	CONTROL	10	34.7	
0	750	99	12.74	± 0.58
0	800	119	12.93	0.59
0	850	111	12.03	0.42
0	900	110	11.69	0.73
15	0	12	32.4	
15	750	87	12.43	0.64
15	800	131	12.45	0.45
15	850	98	11.01	0.33
15	900	96	10.96	0.56
30	0	12	42.2	
30	750	98	13.89	0.35
30	800	97	13.89	0.51
30	850	85	12.64	0.67
30	900	79	12.44	1.07
45	0	9	41.8	
45	750	34	16.08	0.74
45	800	41	16.08	1.24
45	850	31	15.63	1.65
45	900	66	12.40	1.10

* ± 1 SE

Figure 29: Splenic Dry Weight Data; Constant Rate Stress. The mean weight per spleen in milligrams is shown for each treatment group at four constant rate dosages. Codes are as shown in the figure legend.


C R
B 15 + C R
B 30 + C R
16
12
8
4
0
SPLEEN DRY WEIGHT (MG)
750
800
850
900
DOSE (R)

DISCUSSION

In general, the present studies demonstrate that a protective effect is displayed in mice which receive a thermal stress prior to irradiation.

PROTECTION FROM COMBINED STRESS

It has been observed in previous work that when mice receive a non-lethal thermal burn to 10 - 15% of the body surface in addition to whole body X-irradiation mortality occurs earlier and is higher than for non-burned mice (Parr et al., 1952; Baxter et al, 1953; Alpen and Sheline, 1954). The findings of the present study differ in that a protective effect is evinced by the combined stress. However, several differences should be noted which tend to prevent valid comparisons. The protective effect is not found when a superficial burn is employed, but only when a more severe 2° - 3° burn (causing 7 - 11.5% mortality) is applied. A second consideration is that the radiation doses employed in the present experiments were chronic in nature (96 hours), whereas most previous experiments entailed acute irradiation (less than 24 hours). The chronic gamma irradiation was delivered either as simulated fallout or at a constant dose rate and the protective effect was of equal magnitude with both. The lethal dose to half the population in 30 days, the $LD_{50/30}$, shows the protective effect quite clearly. For example, the LD50 estimate for mice subjected to irradiation at a constant dose rate for 96 hours is 852 R, but for mice receiving a 2°

burn (30 second exposure) prior to irradiation the estimate is 1906 R. Thus, to negate the protective effect of the burn the dose of gamma radiation must be increased by 18%.

Table 15 shows the biological effectiveness of chronic gamma irradiation relative to acute X-irradiation (Furo, 1966). The relative biological effectiveness for radiation delivered as simulated fallout over 96 hours is only 0.56, while the RBE for constant rate radiation is 0.50. Thus fallout is the less effective of the two chronic deliveries on the basis of relative biological effectiveness using the LD50 estimates.

The effectiveness of chronic irradiation is markedly altered by the addition of a prior thermal stress. The protective effect of a 30 second exposure in terms of relative biological effectiveness is shown by the decrease from 0.56 to 0.49 in the case of fallout irradiation, and 0.50 to 0.50 with constant rate irradiation. A 2° - 3° thermal burn decreases the lethal effectiveness of chronic irradiation by 7 - 9%; it decreases the sensitivity of mice to the radiation. On the other hand, a 1° burn increases radiosensitivity to constant rate irradiation by 11%. The difference between fallout and constant rate irradiation is probably due to the fact that with fallout most of the dose is delivered in the first day and the dose rate is less than that for constant rate irradiation for half of the 96 hours. This could explain why fallout is less effective than a constant rate delivery in causing lethality. Baker et al (1961) found the same relationship between fallout and

TABLE 15

RELATIVE BIOLOGICAL EFFECTIVENESS (30 DAY MORTALITY)

RADIATION-STRESS	LD50 (R)	95% CL(R)	RBE*
250 kVp X-rays:**			
Acute***	506	494 - 518	1.00
^{60}Co gamma rays:			
Fallout	911	897 - 925	0.56
B.15 + F	942	918 - 967	0.54
B.30 + F	1038	1019 - 1056	0.49
Constant rate:	852	838 - 866	0.59
B.15 + CR	713	685 - 742	0.71
B.30 + CR	1006	986 - 1025	0.50

* relative to 250 kVp X-rays, arbitrarily set at unity

** HVL, 1.33 mm. Cu

*** Acute dose rate; dose delivered in less than 24 hours. (in this case, less than one hour).

slow fallout (constant rate) irradiation, the LD50 estimates for mixed populations being 1109 R and 1008 R respectively. The slopes of the probit linear regressions were also different for the two deliveries, being 14.2 and 10.0; in the present study the slopes are 8.77 and 7.59 for fallout and constant rate irradiation. There appears to be a slight difference between fallout and constant rate irradiation; the latter is the more damaging type of delivery. This could account for the synergistic effect found when a superficial 1° burn (15 second exposure) is combined with a constant rate dosage. The superficial burn is below the threshold required to activate the protective mechanism(s) in the body, so it probably acts to increase the stress on systems critical for survival.

The linear regressions for all treatment groups are essentially parallel to each other which would suggest that the same mechanism(s) is responsible for the development of lethal damage for each combined stress treatment (Lawrence, 1966). The degree of burn would alter the degree of response, not its nature. The groups receiving a prior 15 second exposure show little deviation from animals given fallout radiation alone while groups given a 30 second thermal stress plus radiation show a great deviation. Therefore it would seem likely that the protective effect only occurs when a burn of sufficient intensity is applied, when a threshold is surpassed activating a protective mechanism(s).

ACTION OF HYPOXIA IN RADIOPROTECTION

A likely candidate for the protective mechanism is hypoxia. Hypoxia is already known to be radioprotective to most cells. Vacek and Sugahara (1967) found that the highest intensity of protection in mice occurred when irradiation was conducted in an environment of 10% oxygen; this correlated with reduced oxygen tensions in many organs such as the spleen. Brown and White (1958) found that anoxia in a number of rat tissues was radioprotective and Hall et al (1966) irradiated HeLa cells in vitro, finding an oxygen enhancement ratio of 1.5 at 0.5 Rads per minute. Jaitha and Oliver (1961) reported that some tissues and cells could become sufficiently anoxic to require 2.5 - 3.0 times the dose to achieve the same effect on normally-oxygenated cells. The effect of low oxygen tensions in a cell for prolonged periods of time acts to change the age distribution of cell populations; more mature cells are known to be more radioresistant. Also, the reproductive life cycle is elongated by hypoxia, with a disproportionate lengthening of the G_1 phase (Bedford, 1970). Terasima and Tolmach (1963) and Little (1968) noted that survival of cells was maximal if they were irradiated in the early post-mitotic G_1 phase or pre-mitotic G_2 phase.

The preceding findings would support the contention that the protective effect is a result of hypoxia in the stem cell pools of cell renewal systems at the time of irradiation. Rapid but reparable cellular lesions are induced by low oxygen tensions (5% O_2) which may be involved in the mechanism of increased

resistance to ionizing radiation (Plomteux et al, 1970). This hypoxia could be caused by the physiological and biochemical effects of burn. Sevitt (1949) reported that there is a slowing of the blood flow in dermal capillaries in the burn area, and Alpen and Cheline, (1954) found dilation of the capillaries, loss of plasma (edema) and a subsequent sludging of the blood. The fluid decrease in the circulatory system causes a decrease in cardiac output, a fall in blood pressure, and hemoconcentration (Keeley et al, 1939), as well as the hemolysis of many erythrocytes by direct heating and by the sludging. This decrease in efficiency of the circulatory system results in an inadequate supply of oxygen to many cells in the bone marrow, the spleen and other organs. The anaesthesia was not considered to have any effect on the hypoxia mechanism as the mice had recovered from it by the onset of irradiation.

DISTRIBUTION OF LETHALITY

The deaths over 30 days are distributed discretely by syndrome, the most significant being the bone marrow syndrome. However, with increasing degree of thermal stress either alone, or in combination with radiation, deaths in the 0 - 6 day period markedly increase. Animals succumbing at this time may do so from the gastrointestinal syndrome because serious functional defects are present in the regenerating mucosal layer which may contribute to lethality. It may be that some other system is the target, such as the reticuloendothelial system (Baker and Valeriote, 1968). Quastler and Zucker (1959)

found that the hematopoietic system is much more radiosensitive than the gastrointestinal system so generally a much higher dose would be required to cause gastrointestinal death than hematopoietic death. Deaths within the first few days are most likely caused by secondary shock from the thermal stress (Allen and Sheline, 1954).

Bacteremic mortality (days 7 - 11) is higher with fallout than with constant rate irradiation which could be accounted for by greater damage to the intestinal epithelium and the reticuloendothelial system by the higher dose rates delivered in the first part of the 24 hour period. This could lead to a greater sepsis by gut bacteria, causing a greater mortality in the 7 - 11 day period coupled with a depressed reticulo-endothelial system.

The greatest number of deaths occur in the 12 - 20 day period indicative of hematopoietic damage, the bone marrow syndrome. It is also in this period that the protective effect of 2° - 3° burns and the synergistic effect of a 1° burn on irradiated animals is manifest. This leads to the postulation that these effects are due to alterations in the radiation sensitivity of the hematopoietic system, the more severe burns exerting a protective influence and the superficial burn a more damaging influence. The protective effect may be based on a pre-conditioning of the system to better withstand the radiation stress. This could be achieved by hypoxia, as discussed earlier, rendering the stem cells (progenitors)

less sensitive to radiation, or by stimulation of some facet of the system to repair radiation damage more efficiently.

The decedent survival time (mean survival time of decedents) shows very little deviation between the various treatment groups until the 3° burn is applied. With this stress the early mortality (days 0 - 6) from the thermal stress itself begins to lower the decedent survival time giving the appearance of an overall shift in radiosensitivity. The fairly constant values of this parameter among treatment groups indicate that the hematopoietic or bone marrow system is the major system responsible for the variations in mortality.

Double and triple peaks are found in the 12 - 20 day period with constant rate treatments. A triple-peak is found in conjunction with the protective effect, and a double peak with the synergistic effect and with radiation alone. This separation of mortality into an early, mid and late bone marrow syndrome may be related to the severity of the burn inflicted. The bone marrow syndrome has never been fully elucidated, but remains at this time a collection of signs and symptoms under one main radiosensitive cell renewal system, the hematopoietic system. A severe 2° - 3° burn may affect individual subsyndromes in the 12 - 20 day period more definitively than a superficial burn or no burn.

BURN-IRRADIATION TIME INTERVAL

The time interval between thermal exposure and gamma

radiation has not generally been considered to any great extent in the past. Usually, a set of experiments will employ a particular interval consistently. This arbitrarily-selected value may range from a few minutes (Baker and Valeriote, 1968) to one hour (Alpen and Sheline, 1954) to several months (Michaelson et al, 1963). In the present experiments, the consistent time interval employed is 3.5 hours. This was determined by a study in which the interval between a 30 second thermal stress and gamma radiation was varied from one hour to several hours; the maximum protective effect was elicited by using an interval of 3.5 hours. This optimum time may be related to the sensitivity of some component(s) of the hematopoietic system. It may also be connected with the detoxification of the anaesthetic, which requires about 3.5 hours.

Additional time interval experiments at different dose rates definitely establish the protective effect as dependent upon the dose rate. When mice are irradiated at 0.16 R per minute for 96 hours following a 30 second burn (2°) there is a marked protective effect evident which is optimal for a 3.5 hour time interval. Mice exposed to a dose rate of 0.5° R per minute for 20 hours display a less marked protective effect optimal at 4 hours, while those exposed to 16.7 R per minute show no protective effect. This is in keeping with the fact that dose rate effects occur only at low dose rates (Thomson and Tourtellotte, 1953).

ENDOGENOUS SPLEEN COLONY ASSAY

The hematopoietic system was definitely implicated in the development of the protective effect and so became the object of further experiments designed to further elucidate the nature of the protective mechanism. Hematopoietic stem cells have been shown with partial shielding of marrow to originate in the bone marrow (Cafrey and Everett, 1966), and, under the stress of radiation, to migrate to the spleen and possibly other sites to form hematopoietic colonies (van Bekkum, 1967). Becker et al (1963) provided indirect support that spleen colonies are clones, each arising from a single stem cell. Till and McCulloch (1964) concluded that the effects of radiation on the colony-forming ability of hematopoietic tissue in mice is closely related to radiation effects on 30 day survival in mice given whole body irradiation. This supports the "Elkind hypothesis" (1961) that the lethal effects of whole body irradiation may be explained in terms of the survival of stem cell proliferative capacity. The ability to form colonies on the spleen was tested in mice exposed to thermal stress and constant rate irradiation stress.

The endogenous spleen colony assay on day 12 reveals a greater number of colonies present on the spleens of animals given 30 and 45 second thermal exposures plus constant rate irradiation than on those exposed to the radiation stress alone. This greater level of production would prevent many deaths that ordinarily would be caused by hematopoietic failure (when

constant rate irradiation was given alone). More stem cells may remain viable after radiation, or a higher percentage of viable stem cells may reach and populate the spleen, or both.

The assay reflects the protective effect but not the synergistic effect. The latter effect may be based on activity in another part of the hematopoietic system, or the basic assumption that there is a direct proportionality between the number of surface colonies and internal colonies may be incorrect. This is quite possible since Wolf and Trentin (1968) reported that the spleen contains at least four types of microenvironments that determine the differentiation of pluripotent stem cells into either erythroid, neutrophilic granuloid, megakaryocytic or eosinophilic granuloid colonies. Therefore, if the ratio of erythropoietic to myelopoietic colonies varies with the degree of thermal stress applied, and these colonies occur in different areas of the spleen, then counting only surface colonies could be misleading. This might explain why the endogenous assay for animals given a 15 second thermal exposure plus radiation does not reflect the mortality result. This treatment increased the mortality over constant rate irradiation given alone, so it would be expected to decrease the number of spleen colonies present if the synergistic mechanism were directly involved in this aspect of hematopoiesis. However, the number of colonies was the same or slightly greater with the 15 second thermal stress.

The D_0 (or D_{37}) value represents the dose of radiation

required to reduce the surviving fraction of cells by a factor of 0.37 on the exponential part of the dose response curve. In the endogenous spleen colony assay all treatment groups have the same value for the D_0, indicating the same degree of cell sensitivity with all thermal exposures. Since parallel survival curves indicate no alterations in cell sensitivity, the shift toward higher dose levels may be interpreted as a progressive increase in the number of colony-forming cells as a result of cellular proliferation (Till and McCulloch, 1964). That is, a 2° - 3° burn exerts a protective effect by increasing the number of stem cells populating the spleen.

The splenic dry weight data display both the protective and synergistic effects seen in the mortality data. The mean dry weight of spleens from mice subjected to a 2° burn and constant rate radiation is consistently higher than for spleens from non-thermally stressed mice. The dry weight for the 1° burn plus radiation treatment is consistently lower than for radiation alone. This may lend support to the earlier proposal that the different degrees of burn stimulate colonies to develop in different microenvironments of the spleen (surface and internal).

BACTERIAL ENDOTOXINS AS RADIOPROTECTIVE COMPOUNDS

Auto-infection caused by Escherichia coli is important in the development of the hemorrhagic syndrome. When E. coli endotoxin is administered to irradiated animals, the syndrome

is sharply increased (Kiselev et al, 1964). Smith et al (1958) have done a good deal of work using the bacterial endotoxins of Salmonella typhosa. They found that if the endotoxin is administered 24 hours before irradiation recovery is slightly increased for lymphocytes and there is a great mobilization of granulocytes. The induction of hematopoiesis is considered responsible for the increased survival. They also discovered that the endotoxin moves ahead the time when effective production of stem cells is resumed (Smith et al, 1966a). If treated one day before irradiation the production begins about 2 - 3 days after irradiation; this would correspond with the last day of the 96 hour chronic irradiation in the present project. Stem cell production is thus resumed earlier under endotoxin stress. This earlier onset of hematopoiesis could account for the radioprotective effect on mortality. If more stem cells were present during radiation more would survive; the same proportion would be eliminated but the absolute number of survivors would be greater. Survival was markedly increased by a single injection of endotoxin before or shortly after irradiation. Bone marrow cellularity and splenic nodules also increased (Smith et al, 1966b).

Tumanyan et al (1970) have very recently discovered a radioprotective effect of a polysaccharide isolated from the somatic O-antigen of Salmonella typhi which apparently acts to stimulate natural immunity and to normalize hematopoiesis more rapidly in animals radiated and protected.

The thymus may play a role in radioprotection that has yet to be elucidated. This organ is necessary for the recovery of the immune system of the body following radiation damage; injected bone marrow cells must obtain their immune competence from the thymus of the lethally irradiated host (Miller et al, 1963). There is a definite influence exerted on bone marrow lymphocytes which enables them to become sensitized to proliferate in the presence of an antigenic stimulus. They then produce a cell-mediated immunity (Turk, 1969). The thymus has also been observed in the present experiments to increase in size following severe thermal stress (2° - 3° burn). This may be indicative of an increased effect on hematopoiesis which could be related to the protective effect. Further studies on the thymus using combinations of thermal stress and irradiation could determine if there is a direct or an indirect effect by this organ on hematopoiesis and the protective effect of burn.

SUMMARY

When thermal stress is applied to mice prior to the onset of chronic gamma irradiation the mortality by day 30 is altered according to the severity of the thermal stress. The gamma radiation was delivered as simulated fallout or at a constant dose rate over a 96 hour period, and the LD^{50}_{30} estimates were 911 R and 852 R respectively. If the mice are pre-stressed with a 30 or 45 second thermal exposure, the LD^{50}_{30} estimate is increased by 14 - 18%. A 15 second thermal exposure does not affect the LD^{50}_{30} estimate for fallout irradiated mice, but decreases it by 16% for constant rate irradiated mice.

Mortality for all treatment groups occurs mainly in the 12 - 20 day time period indicative of hematopoietic failure. The fluctuations in mortality arising from the application of various thermal burns before irradiation are observed primarily during this period and are therefore related to alterations in damage to the hematopoietic system. The protective effect of a 30 or 45 second burn is reflected in the 12 - 20 day period by a decrease in the mortality from irradiation. For these protective burns the early mortality is increased but the overall mortality by day 30 shows a decrease.

The time interval between a 30 second thermal stress and irradiation affects the amount of protection afforded; a maximal protective effect is obtained by using a 3.5 hour interval. Also, with increasing dose rate the protective effect gradually disappears.

The endogenous spleen colony counts support the protective effect of a 30 second burn on gamma irradiated mice. The D_0 values are identical for the survival curves (about 130 R). The mean dry weights of the spleens also exhibit trends which reflect the protective mortality effect.

The combination of a 30 second thermal stress and a 96 hour chronic gamma irradiation dosage results in a protective mortality effect on irradiated mice. This is partitularly evident in the 12 - 20 day time period, characteristic of the bone marrow syndrome. The number of spleen colonies is also increased with this combination, indicative of enhanced hematopoietic recovery.

GLOSSARY

Anoxia ---------- No O_2 in the tissues; hypoxia is a more correct term as there is rarely a complete absence of O_2.

B ---------- A thermal exposure; e.g. a 15 second exposure is B.15.

CR ---------- Gamma radiation delivered over a time course at a constant dose rate.

Denudation ---------- Loss of the protective cell layer covering the intestinal villi (by radiation).

ESC ---------- Endogenous Spleen Colony.

Electronic equilibrium -- Ions produced within the volume of air in the probe lose all their energy within it.

Endogenous colonies ----- Formed from the animal's own stem cells.

Endotoxin ---------- Product derived from the breakdown of bacteria.

Erythropenia ---------- Deficiency of erythrocytes.

Erythropoietic ---------- Concerning the maturation of red blood cells (erythrocytes).

Exogenous colonies ------ Formed from the donor's stem cells.

F ---------- Simulated fallout radiation.

Functional cell ---------- The most mature cell of a cell renewal system, responsible for carrying out the function of that system.

Granulocytopenia -------- Deficiency of granulocytes.

Hematopoiesis ---------- Formation of blood cells (hemopoiesis).

Hematopoietic ---------- Concerning hematopoiesis.

Heteromorphogenic division Division of a stem cell to form one stem cell and one cell destined for the functional pool.

Homomorphogenic division -- Division of a stem cell to form two stem cells.

Hypoxia ---------- Low O_2 tensions in tissues.

LET ---------- Linear energy transfer; the energy lost per μ of path length of the primary ionizing particle.

LD_{30}^{50} ---------- The dose of radiation causing death of one half the total number of animals exposed within 30 days.

Myelopoietic ---------- Concerned with formation of the myeloid series of blood cells. (granulocytes).

Necrosis ---------- Breakdown of tissue leading to formation of an exudate and a scar.

Pluripotent cell ---------- Capable of becoming one of several functional cell types.

R ---------- Roentgen; the basic unit of radiation exposure defined in terms of the amount of ionization produced in a given mass of air.

Rad ---------- Roentgen absorbed dose.

RBE ---------- Relative biological effectiveness; the inverse ratio of doses of different quality radiations that produce the same effect on the tissue or organism.

Reticuloendothelial system ---------- Cells throughout the body that are phagocytic for all types of foreign particles (macrophages).

SE ---------- Standard error.

Stem cell ---------- The youngest or earliest cell in a cell renewal system that is capable of self-reproduction.

Supralethal dose ---------- A dose larger than the LD100.

Synergistic effect -------- An effect of two combined stresses that exceeds the sum of the two individual stresses.

Thrombopenia ---------- Deficiency of platelets.

1°, 2°, 3° burns ---------- First, second and third degree burns. The degree of burn increases with depth of thermal damage.

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