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PROTECTION AGAINST IONIZING RADIATION

X-irradiated Monkeys Receiving Preirradiation Prophylaxis and Postirradiation Therapy

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FOREWORD

This report was prepared in the Bionucleonics Department* and the Veterinary Services Branch† by the following personnel:

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In this study, postirradiation symptomatic therapy of the radiation syndrome has been utilized to provide significant extension of survival time, compared to unirradiated controls, of mice and female primates irradiated to a total dose of 900 r. Further, the therapeutic regimen has been successfully combined with chemical radioprotectors to further prolong the survival time in a third group of animals. This combined treatment is very effective than the therapy alone. The overall clinical condition of these animals is far superior to that of the untreated irradiated animals. Pathologic studies of the treated animals indicate that the two major causes of death were bone marrow failure and severe lymphoid atrophy. The protective chemical treatment is in itself a combination of different mechanisms of radioprotection, routes of administration, and rates and sites of absorption of the drugs.

This technical documentary report has been reviewed and is approved.

Robert E. Payne
Colonel, USAF, MSC
Chief, Operations Division

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1. INTRODUCTION

In previous studies in this laboratory it has been shown that the radioprotective conferred by certain sulfhydryl-containing chemicals can be enhanced in rate, either additively or synergistically, by other compounds (2, 3). Similar studies have also been effected in monkeys, and the findings on the primate substantiate those found for the rabbit (4).

At the present "state-of-the-art" of chemical radioprotection, it would seem that, although effective radiation dose reduction can be achieved, a supraoptically irradiated animal will still sustain appreciable damage. Some, if not all, of the clinical sequelae of radiation sickness thus will be manifested. Clinical treatment of chemically protected animals therefore becomes essential if the dose-reducing advantages of the radioprotective chemicals are to be optimized.

This report details the first study accomplished at this laboratory in which radioprotective treatment was followed by medical treatment after the irradiation had been administered. Eight monkeys were given the best chemical treatment available in this laboratory (2, 4, 5), after which they received 99% or 100% lethal cranial exposure of 6 Gy at this laboratory, and approximately 7.5 Gy at the Institute for Cancer Research. The results indicate that the combination of treatments significantly enhanced the bioprotectency of all survivors, the longest lived surviving at 618 days postexposure.

2. MATERIAL AND METHODS

The monkeys used were of the Modern strain of rhesus monkey and ranged from 4.5 to 5.5 lb. The eight treated animals were chosen at random from a larger group of healthy animals. In this selection, no distinctions were made on the basis of sex; these were two females and six males. Ten additional animals (four females and six males) were irradiated at 50 Gy; controls; four preirradiation controls, and six postirradiation therapy controls.

The whole-body irradiations were performed with a Picker X-ray machine at 3.60 kVp, 15 cm, with 1 mm, all and 0.25 mm. Cu added filtration. The dose rate was 28 to 29 r/min; the exposure cage was located at 15 cm. 5,2-Aminothiazole dibromide (ABT) was synthesized and purified in this laboratory by the method of Stargard (2).

All protected animals received ABT-dibromide (75 mg/kg, body weight) plus cephalin-NCI (25 mg/kg, body weight intravenously, 60 minutes prior to irradiation). ABT-dibromide (150 mg/kg, body weight) plus cephalin-NCI (150 mg/kg, body weight) orally, 15 minutes before irradiation, and Mualinal (8 to 10 mg/kg, body weight) for anesthesia, 15 minutes prior to irradiation.

Intravenous doses of ABT and cephalin were prepared by weighing the appropriate amount of each drug, reconstituting, and infusing them with 2.5 normal NaCl to a final pH of
7.2 to 7.4. Samples were diluted to 10 ml. with distilled water and administered 60 minutes before irradiation.

Oral doses of AET and cysteine were prepared in a similar manner, neutralized together with 2.5 normal NaOH to a pH of 7.2 to 7.4, diluted to 8 to 10 ml. with 0.5 M phosphate buffer, and gavaged 15 minutes before irradiation.

Nembutal was administered intravenously 15 minutes before irradiation at a dose of 8 to 10 mg/kg body weight. A minimum dose was used to achieve anesthesia, checking by the "eye-blink" reflex method. As a result, the entire calculated dose was sometimes not used.

Hematologic examinations were accomplished on blood drawn by femoral puncture; the methods specified by Wintrobe (9) were used for counting and for other determinations.

Bacteriologic and parasitologic examinations were effected on blood and feces both before and after irradiation. Samples for parasitologic were examined by the ether concentration method; stool bacteriologic samples were examined by the standard "three-media" technic and then by tetraphenylphosphine enrichment; blood bacteriologic samples were collected on 1-oz. agar slants using tryptose-glucose broth, and then plated by standard technic. To aid in the case of antibiotic, positive blood cultures were tested for sensitivity to various drugs by using the paper disc method.

Temperatures were obtained rectally in this laboratory. 102°F is a normal mean value for this strain of monkey.

### Table I

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Survived thru (days)</th>
<th>Experimental Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal A</td>
<td>0</td>
<td>Non</td>
</tr>
<tr>
<td>Animal B</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Animal C</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Animal D</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Animal E</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Animal F</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Animal G</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Animal H</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Animal I</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Animal J</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Animal K</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal L</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal M</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal N</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal O</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal P</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal Q</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal R</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal S</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal T</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal U</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal V</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal W</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal X</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal Y</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Animal Z</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

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Water intake was obtained by calculating the amount consumed from a 500-ml bottle of water given to each animal daily. Food consumption (in calories) was calculated by estimating the unconsumed portion of the amount given approximately 16 hours after feeding.

The survival time, calculated in days, was used in comparing the treated animals with the irradiated-therapy controls, and separately with the irradiated controls that received no treatment. The Mann-Whitney U Test was used, as expounded by Siegel (1956). Survival data were treated in contingency tables and the probabilities were calculated by Fisher's Exact Method at the 30-day point.

3. RESULTS

Table I summarizes the longevity of the eight animals that were treated before and after irradiation (group II); control animals that were given therapy after irradiation (group III); and the four irradiated control animals that received no treatment (group I). The survival time for group I was 10 days; group II, 31 days; group III, 135 days.

Figure 1 shows weight and temperature changes for the first 30-day period postirradia
tion. These values are means for the animals which were alive when the sample was taken; weights have been presented as percentages of the baseline values before irradiation and, in turn, have been normalized to the normal value for the rhesus monkey in this colony. The animals pretreated with the chemotherapeutic agents (group III) maintain their weight better than those that received additive treatment only (group III).

Variations in temperature, however, animals in group III do maintain their body temperature better during the early and late stages of the 30-day period, and there is less fluctuation than in group II. The temperature in group III is a characteristic which persists for a period of time after the treatment is completed. These trends are marked for the animals alive at the time of the sampling. There are very few differences in the two groups. The

| TABLE II |
| Statistical probabilities for the survival of monkeys |

<table>
<thead>
<tr>
<th></th>
<th>Fisher's Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mann-Whitney &quot;U&quot; Test</td>
</tr>
<tr>
<td></td>
<td>(days until death)</td>
</tr>
<tr>
<td>Group I vs. II</td>
<td>.035  1  &gt;  .05</td>
</tr>
<tr>
<td>Group I vs. III</td>
<td>1.00     1.00</td>
</tr>
<tr>
<td>Group II vs. III</td>
<td>1.00     1.00</td>
</tr>
<tr>
<td>Group III vs. III</td>
<td>1.00     1.00</td>
</tr>
</tbody>
</table>

White blood cell counts are higher for group II and although the less extended platelets and reticulocytes are also higher in group III, hemoglobins are somewhat higher during the last week.

Table II shows the results obtained by applying Fisher's Exact Test and the Mann-Whitney "U" Test to these data. None of the differences is considered significant (rejection probability P > 0.05); however, there is significant improvement in survival times in all cases.

Table III gives an overview of the clinical history of the group II before and after irradiation. The animals have been listed in order of increasing mortality. The numbers in parentheses indicate the day on which they were positive, and the days on which they were administered. It will be noted that animals receiving the best treatment lived the longest. Clearly, however, these animals showed the most symptoms since symptoms were the basis for therapy.

As a general overview of the animals of group I was very poor, they were delineated quite easily from the treated groups at the end of the first week. Symptoms included
<table>
<thead>
<tr>
<th>Animal</th>
<th>Preirradiation</th>
<th>Postirradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

Clinical summary for group IV

<table>
<thead>
<tr>
<th>Animal</th>
<th>Ova and parasites</th>
<th>Enteric pathogens</th>
<th>Pathogens</th>
<th>Hematology</th>
<th>Ova and parasites</th>
<th>Enteric pathogens</th>
<th>Pathogens</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>92E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
<td>Negative</td>
<td>Nitella (9)</td>
<td>Staphylococci</td>
<td>ampicillin (1)</td>
<td>Blood transfusion (19), Stroclin (10)†</td>
</tr>
<tr>
<td>93E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
<td>Negative</td>
<td>Negative</td>
<td>Enterococci</td>
<td>ampicillin (11)</td>
<td>Lomonal and saline intravenously (12), Stroclin (12)</td>
</tr>
<tr>
<td>95E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
<td>Negative</td>
<td>Staphylococci</td>
<td>ampicillin (13)</td>
<td></td>
<td>Lomonal MB and 5% dextrose intravenously (14), Rejected (15)</td>
</tr>
<tr>
<td>97E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
<td>Negative</td>
<td>Staphylococci</td>
<td>ampicillin (14)</td>
<td></td>
<td>Lomonal MB and 5% dextrose intravenously (15), Achromycin (16-18), Amine-plus, Lomonal MB and 5% dextrose intravenously (17-18), Rejected (18)</td>
</tr>
</tbody>
</table>

**Notes:**
- Figures in parentheses indicate the number of days for therapy.
- All drugs given intravenously except as noted.
- These letters are the initial part of the name of the drug used.
### Table IV
**Clinical Summary for group III**

<table>
<thead>
<tr>
<th></th>
<th>Preirradiation</th>
<th>Postirradiation</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal No.</strong></td>
<td><strong>Ova and parasites</strong></td>
<td><strong>Enzyme pattern</strong></td>
<td><strong>Feces</strong></td>
</tr>
<tr>
<td>83E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
</tr>
<tr>
<td>125E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
</tr>
<tr>
<td>99E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
</tr>
<tr>
<td>43E</td>
<td>Negative</td>
<td>Negative</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate days postirradiation that symptom was noted or drug administered.

†All drugs given intramuscularly unless otherwise indicated.

‡These letters are an integral part of the name of a class of Abbott parenteral fluids indicating clinical use.
acute anorexia, diarrhea, bloody diarrhea, dehydration, some epilation, and very low body temperature occurring just before death. At the end of the first week, essentially no white cells or platelets were detected in the peripheral blood. The extremely short survival time of the monkeys in group I makes it impossible to plot curves showing data comparable to that of the other groups.

Figure 3 shows the calorie and fluid intake of the animals expressed as the group mean percent of the amount offered. The numbers in parentheses represent the number of animals comprising the group at that time. These data indicate that failure to utilize adequate fluids is an extremely important factor in maintaining the normal fluid balance, and that the protective drugs do not appreciably alter the situation. Postirradiation anorexia was seen in both groups, but was somewhat less severe in group III and appeared some seven days later than in group II.

Tables V, VI, and VII describe the pathologic findings for groups I, II, and III, respectively. Bone marrow failure and atrophy of lymphoid tissue are consistent findings in all groups. Animal 48E (group III), which lived 648 days after irradiation, actually showed a slightly hypercellular bone marrow with numerous mitotic figures. It is interesting to observe the occurrence of meningitis (99E, table VII); this finding has been noted in several animals in other phases of the research (60).

2 DISCUSSION

This is the first experiment at this laboratory in which any protected animal has survived as long as 30 days after being given...
### TABLE V
**Pathologic summary for group I**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Gross findings</th>
<th>Microscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>13F</td>
<td>Petechial hemorrhage of skin, focal hemorrhage of subarachnoid space, cerebral cortex, lung.</td>
<td>Atrophy, bone marrow and lymphoid tissue. Slight atypia of colonic mucosa.</td>
</tr>
<tr>
<td>14E</td>
<td>Ulceration and hemorrhage, cecum, descending colon and rectum. Petechial hemorrhages, mucosa, stomach, urinary bladder.</td>
<td>Atrophy, bone marrow and lymphoid tissue. Fatty metamorphosis, slight, liver.</td>
</tr>
</tbody>
</table>

### TABLE VI
**Pathologic findings for group II**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Gross findings</th>
<th>Microscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>78E</td>
<td>Petechial hemorrhages of skin, serous surfaces, epidermis, liver, stomach, colon, urinary bladder, ileum. Ulceration and hemorrhage, rectum.</td>
<td>Atrophy, bone marrow, with early regeneration. Atrophy, lymphoid tissue.</td>
</tr>
<tr>
<td>114E</td>
<td>Softening of right maxilla. Purulent material in maxillary sinuses.</td>
<td>Reduced cellularity of bone marrow. Atrophy, lymphoid tissue. Osteomyelitis, maxilla, acute and chronic.</td>
</tr>
</tbody>
</table>
### TABLE VII
Pathologic findings for group III

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Gross findings</th>
<th>Microscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>82E</td>
<td>Edema and congestion, colon, slight.</td>
<td>Crypt abscesses, colon. Atrophy, lymphoid tissue. Hypoplasia of bone marrow.</td>
</tr>
</tbody>
</table>

900 r of x-rays, it is the first time a symptomatically treated animal has survived more than 20 days at this level of irradiation.

The chemical treatment used for radioprotection is more extensive than other treatment previously reported in primates (11, 4). We combined different mechanisms of protection, routes of administration, and sites of application. For example, (1) the intravenous dose affords a high blood level of a good radical sump (AIC or a homolog) and an additional compound (cysteine) which may be a radical sump and may also participate in normal oxidative metabolism; (2) an oral dose of the same compound provides direct physical contact and presumably absorption in the gastrointestinal tract, and allows the total concentration of the drugs to be increased without noticeably increasing the toxicity; and (3) the
use of pentobarbital sodium introduces a drug which decreases respiration (in terms of relative \( O_2 \) concentrations), lowers the overall metabolism rate, creates some tissue hypoxia, and potentiates the effect of AET (5).

The actual chemical species present in a homogeneous aqueous mixture of AET and cysteine is yet to be determined. Preliminary work shows that the protective effect of this mixture depends on the concentrations of the two compounds; it also indicates that when the two compounds are mixed in water before treatment, they are more effective than when they are given to the same animal in separate aqueous solutions. The product may be a mixed disulfide or a sulfhydryl compound of relatively high molecular weight. The problem is being investigated by paper chromatographic methods.

REFERENCES


USAFA School of Aerospace Medicine, Brooks AF Base, Tex.


Unclassified Report.

In this study, postirradiation symptomatic therapy of the radiation syndrome has been utilized to provide significant extension of survival time, compared to irradiated controls, of Macaca mulatta primates x-irradiated to a total dose of 100 r. Further, the

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