Protection and Recovery Discussion Meeting

Abstracts presented at the Palmer House, Chicago, Ill.
April 13, 1964

Chairman: R. A. Popp

Observations on Dimethylsulfoxide

Victor Di Stefano. From the University of Rochester, Rochester, N. Y.

Following the i.v. administration of dimethylsulfoxide (DMSO) to cats, dimethylsulfide was noted on the expired breath within 1 minute. Single i.v. doses of 200 mg./Kg. DMSO produced apnea and a transient fall in blood pressure. Hemolysis resulting in hemoglobinuria and methemoglobinuria was also observed. I.p. administration of DMSO as well as dilution of DMSO with isotonic saline prior to i.v. administration reduced hemolytic activity.

Further Studies of Leukocytotoxic and Hemagglutinating Isoantibodies in the Dog

S. Kasakura, P. Rubinstein and J. W. Ferrebee. From the Mary Imogene Bassett Hospital (Affiliated with Columbia University), Cooperstown, N. Y.

As reported previously, cytotoxic isoantibodies and hemagglutinins were produced by repeated grafts of skin and repeated injections of marrow, lymph node, and splenic tissue. There was dissociation in the cytotoxic and hemagglutinating activities in sera drawn at various intervals following immunizations. No direct relationship was found between the relative strength of the two activities. When a panel of antisera was used to type 40 dogs, the frequency of cytotoxic activity in some sera was greater than the frequency of hemagglutinating activity; the converse was true in other sera. These findings suggest that the antibodies causing hemagglutination are not necessarily the same ones that cause cytotoxicity. Efforts were made to make the cytotoxic isoimmune sera specific for certain antigens or groups of antigens in order that dogs may be typed for the presence or absence of these antigens. For this purpose the immune sera were absorbed with the leukocytes of selected dogs. Absorption by positively reacting leukocytes removed cytotoxic activity against the leukocytes of some dogs and left it for others. This reactivity in turn could be removed by absorption with the positively reacting leukocytes of other dogs. A limited study of absorption of two antisera has indicated the existence of a number of isoantigens in canine leukocytes. A similar study of the absorption of hemogglutins has led to similar conclusions. Many isohemagglutinins could be absorbed by leukocytes as well as by erythrocytes.

Quantitative Comparison of the Ability of Various Transplanted Tissues to Initiate Antibody Formation in the Cyclophosphamide-Treated Mouse

George W. Santos and Albert H. Owens. From the Department of Medicine, Johns Hopkins University, Baltimore, Md.

A modification of an in vivo tissue culture technic for quantitating antibody production (Makinodan et al.) was developed utilizing pretreatment with a single dose of cyclophosphamide (CY). B6D2F1/J female mice, 10–12 weeks old, were given 300 mg./Kg. of CY i.p. At various times thereafter, 11.3 x 10^6 isogenic spleen cells were injected i.v., and 1 ml. of 1 per cent sheep red cells injected i.p. Animals were bled 7 days later and agglutinin titers determined (Log_2/dilution). The data indicated that the im...
muno-suppressive activity of CY had disappeared after 1 hour. Mice were given graded doses of various isogeneic tissues i.v., together with 1 ml of 1 per cent sheep red cells, 4 hours after they had received 300 mg./Kg. of CY i.p. Agglutinin titers were determined 7 days later. When the titer was plotted against the dose (log.) cells injected, linearity was obtained with a slope of about 1. The ability of spleen cells from BALB/cJ, C3H/B, C57BL/6J, and DBA/2J mice to initiate a primary response in the B6D2F1/J host did not appear to differ from isogeneic cells. Tentatively, the relative potency of the various tissues to initiate an agglutinin response is indicated in the following table.

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>No. Cells (x 10^6) to Produce Mean Titer of 5.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood*</td>
<td>1</td>
</tr>
<tr>
<td>Blood</td>
<td>3</td>
</tr>
<tr>
<td>Spleen*</td>
<td>3</td>
</tr>
<tr>
<td>Spleen</td>
<td>9</td>
</tr>
<tr>
<td>Lymph node</td>
<td>12</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>48</td>
</tr>
<tr>
<td>Thymus</td>
<td>384</td>
</tr>
</tbody>
</table>

*Animals immunized 6 weeks previously with 1 ml. of 10 per cent sheep red cells.

**POSSIBLE HYBRID-SUBSTANCE INTERACTION EFFECT IN BONE MARROW TRANSPLANTATION**

Don H. Shaw, Lois W. Jenkins and Kelly H. Clifton. From the Radiology Research Laboratories, Department of Radiology, Medical School, University of Wisconsin, Madison, Wis.

Male rats, 12-14 weeks old, of the W/Fu strain were subjected to lethal total-body irradiation (1200 r at 7.4 r/min.) from a Co137 source and received bone marrow transplants intracardially 24 hours after the irradiation as follows: (1) BN-CC Marrow, bone marrow from male rats of the BN strain known to be homozygous for the erythrocytic antigenic factor C; (2) BN-CD Marrow, bone marrow from male rats of the BN strain known to be heterozygous for the erythrocytic antigenic factors C and D; (3) BN-DD Marrow, bone marrow from male rats of the BN strain known to be homozygous for the D factor; and (4) Tyrode solution without any marrow, as untreated controls.

A comparison of the per cent of 30-day survival and the average day of death for those dying in <30 days for the four treatment groups is shown in the table at the end of this abstract.

Note: The W/Fu inbred strain is homozygous for the D factor and differs from the BN strain at the R1 histoincompatibility locus. Though the BN strain is segregating for the C and D antigenic system, members within this strain do not manifest any demonstrable histoincompatibility between the individuals of the three genotypes CC, CD, and DD.

It is apparent from the data that groups 1 and 3, homozygous CC and DD marrow transplants, had significantly better 30-day survival than either the controls or the heterozygous CD marrow group. The data also show a significant difference in average day of death for those dying before 30 days for groups 1 and 3 (CC and DD marrow) in contrast to group 2 (CD marrow), indicating a different mode of death. There are several hypotheses which might be employed to reconcile the therapeutic superiority of homozygous marrows, either CC or DD over that of heterozygous CD marrow. (1) It has been suggested that the heterozygous CD marrow was more prolific due to a heterotic effect; therefore, the donor vs. host reaction was more profound. (2) Another suggestion might be that CC and DD animals were genetically more different from the CD animals than just their differences with respect to the C and D antigens. (3) A third suggestion was that the CD marrow possessed...
PROTECTION AND RECOVERY DISCUSSION MEETING

An antigenic interaction specificity not found in either homozygous class and that this factor elicited a stronger host rejection of the donor marrow. That is, a “hybrid-substance” associated with the heterozygous state of the C and D alleles, and that this antigenic specificity was adequate to provoke a host vs. donor immunologic response.

Several things tend to reduce the plausibility of a heterotic effect: (1) chimera formation was 71.4 per cent for the CC marrow group and only 58.8 per cent for the CD marrow group (no information on chimerism for the DD group since here the host and donor cells are indistinguishable, both being DD); (2) Fe59 was injected with bone marrow suspensions and uptake and subsequent labeling of red cells was faster in the CC group than in the CD group; (3) CD spleen cells would be expected to be more effective than CC spleen cells in a donor vs. host reaction and the indications were the opposite; that is, the CD spleen transplantees survived slightly longer than those hosts transplanted with CC spleen cells.

With respect to postulate 2, a greater genetic difference was found than that indicated by the antigenic factors C and D; (1) the BN animals used were obtained at the same time from the same colony; many of the CC, CD, and DD individuals were segregants from the same litter; (2) since these animals are inbred and show no demonstrable histoincompatibility differences among themselves, one would expect that any factor or factors not associated with the CD locus would be randomly distributed with respect to the three possible blood types. Therefore, although a “hybrid-substance” effect seems improbable, it is at least more plausible than either heterosis or a genetic difference not associated with the CD system. Further studies are under way to elucidate this phenomenon.

<table>
<thead>
<tr>
<th>Donor Marrow Type</th>
<th>Per Cent 30-Day Survival</th>
<th>Average Day of Death for Those Dying in &lt;30 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN-CC</td>
<td>57.1</td>
<td>24.7 ± 1.6</td>
</tr>
<tr>
<td>BN-CD</td>
<td>0.0</td>
<td>12.7 ± 2.8</td>
</tr>
<tr>
<td>BN-DD</td>
<td>37.5</td>
<td>21.8 ± 5.2</td>
</tr>
<tr>
<td>Controls</td>
<td>6.25</td>
<td>15.1 ± 4.8 (the 1 survivor died on day 37)</td>
</tr>
</tbody>
</table>

COMPETITIVE GROWTH OF HEMATOPOIETIC CELLS IN LETHALLY IRRADIATED MICE

R. A. Popp and D. M. Popp. From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

When mixtures of bone marrow cells from each of two strains of mice are injected into a lethally irradiated mouse, the recipient’s hematopoietic system is repopulated by one of the two types of donor marrow (Ann. New York Acad. Sc. 114:538, 1964). Which of the two types of bone marrow continued to function depended upon the strains of origin of the donor marrows and the strain of the recipient. The strain of origin of the hematopoietic cells that repopulate the recipient was determined from analysis of the red cell hemoglobin or serotype. The relative efficiencies of bone marrow and of fetal liver from additional strains of mice to grow in F1 and homologous recipients were determined. The results suggested a kind of hierarchy of efficiencies among the strains studied. Of primary importance, hematopoietic cells from strain 101 and C3H (H-2k) mice were more efficient than those from BALB/c, DBA/2, and B10.D2 (H-2d) mice, which were more efficient than marrow cells from A.BY, C57BL/6, and C57BL (H-2b) mice. Secondly, when marrow cells of the same H-2 type were used, erythrocytes from both types of donor marrow may be found in the recipients at 60 days after treatment; however, proliferation of one of the donor marrows ceased within 180 days after marrow transfer. The decreasing order of efficiency of donor marrows of similar H-2 types was as written above. Fetal liver hematopoietic cells also showed marked differences in their abilities to repopulate lethally irradiated recipients. The hierarchy for fetal
liver cells was similar to that for bone marrow. These studies confirm and extend our earlier findings that the relative abilities of hematopoietic cells from different strains of mice to repopulate lethally irradiated recipients is to a large extent, but not solely, H-2 associated.

**Density Gradient Centrifugation of Bone Marrow**


Attempts were made to separate bone marrow cells by gradient centrifugation in a sucrose gradient and to identify cells vital for protection against total-body irradiation and those responsible for graft vs. host reactions. Using a sucrose gradient between 17 and 50 per cent in a siliconized centrifuge tube, 15 ml. capacity, mouse bone marrow cells were layered over the sucrose and spun in a cold centrifuge (4 C.) at 100 x g for 12 minutes. These conditions were optimal for separation of cells in sucrose. They were derived by varying the speed of centrifugation, the volume and specific gravity of sucrose, the number of marrow cells, and the volume of cell suspension.

Leukocyte counts and smears were made of each grossly visible layer of cells (4-5 layers) after washing cells in 15 volumes of isotonic saline. The results indicate a wide distribution of white cells in the gradient with larger cells settling in the denser sucrose layers. The method does not permit a clear morphologic separation of marrow cells. By incubating marrow in serum 199 and tritiated thymidine prior to centrifugation it was possible to demonstrate concentration of cells with the label in the denser layers of the gradient. A preliminary experiment demonstrated the toxicity of 50 per cent sucrose for marrow cells. Concentrations of sucrose from 17 to 35 per cent did not destroy the protective capacity of marrow cells for irradiated mice. Cellular layers were removed from the gradient, rinsed in isotonic saline, and injected into isologous and homologous mice 24 hours following an LD99 of total-body X-irradiation. Isologous mice each received a minimum protective dose of marrow cells (500,000). In several experiments, certain of the cellular layers protected irradiated isologous hosts better than an equal number of whole marrow cells although no consistency was noted. The viability of separated cells was also demonstrated in homologous hosts given 5 million cells. Further work is in progress to study the protective effects of such cells and to detect any advantage over an equal number of whole bone marrow cells in irradiated hosts.

**Differentiation of Hematopoietic Stem Cells**

Jerry P. Lewis and Frank E. Trobaugh, Jr. From the Department of Medicine, Presbyterian-St. Luke’s Hospital, and the Department of Medicine, University of Illinois College of Medicine, Chicago, Ill.

In 1963, Becker, McCulloch, and Till published data demonstrating that the hematopoietic spleen colonies resulting from the infusion of marrow into lethally irradiated mice were true clones, each developing from a single cell. We shall show that this cell develops into mature marrow elements. We have previously shown that during colony growth this cell divides, producing additional colony-forming cells. Thus, this cell from which each colony develops has characteristics of a stem cell. That is, this cell produces mature marrow elements, at the same time maintaining its own primitive precursor state. Detailed microscopic studies were undertaken to learn something about the differentiation of murine stem cells. Following irradiation with 750 r, 13-14 week-old CAF1/Jackson female mice were injected with isologous hematopoietic tissue in numbers sufficient to yield approximately 10 to 20 colonies per spleen. The spleens were removed and fixed in formalin 9 days later. The formalin-fixed spleens used in this study were serially sectioned at 4 μ and every fifth section mounted and stained with...
PROTECTION AND RECOVERY DISCUSSION MEETING


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PROTECTION AND RECOVERY DISCUSSION MEETING


Our studies of the differentiation of hematopoietic stem cells obtained from marrow have been described earlier. We have subsequently performed similar studies on stem cells obtained from blood.

In 1930, Woenckhaus showed that if one
parabiotic rat were exposed to lethal radiation both animals survived. It now seems evident that the recovery of this irradiated animal was effected by the seeding of blood-borne hematopoietic stem cells supplied by the shielded animal. Twenty years later, Brecher and Cronkite verified the protective effect of preirradiation parabiosis and in addition showed that parabiosis accomplished immediately postirradiation provided protection. In 1956, Congdon, Smith, and their co-workers established the therapeutic value of iv injected leukemoid blood in lethally irradiated mice, and since that time others at the Oak Ridge National Laboratory have demonstrated that immunologically competent cells and hematopoietic stem cells capable of repopulating marrow spaces circulate in the peripheral blood of mice.

Our results indicate that: (1) hematopoietic stem cells circulating in blood differentiate in the same manner as do those from marrow; (2) stem cells occur in the marrow 60 to 100 times as frequently as in the blood; (3) stem cells of the blood produce independent colonies of developing megakaryocytes, and (4) hematopoietic stem cells in the blood like those from marrow tend to be unipotential in their differentiation, or if pluripotential, are directed at the time of seeding to differentiate along one cell line. All studies have been performed using the spleen colony technic in 13–14 week-old CAF1/J female mice.

The population density of hematopoietic stem cells in blood and in marrow has been estimated. Approximately five colonies were formed for every 10,000 injected marrow cells and approximately five colonies were formed for every 500,000 to 1 million injected blood leukocytes. These data may be analyzed in either of two ways. Using the parallel line assay, which assumes that the number of colonies formed is an exponential function of the number of cells injected, it is estimated that the population density of stem cells in the marrow is 100 times that of blood. If we assume that the number of colonies formed is a linear function of the number of stem cells injected, it is estimated that the population density of stem cells in the marrow is approximately 60 times that of blood.

Sufficient pooled isologous peripheral blood was injected into lethally irradiated mice to form approximately 10 colonies per spleen. At 9 days the mice were killed, and the spleens sectioned and studied by the technics described earlier by Dr. Lewis. The size, location, and cellular makeup of 100 colonies formed from leukocytes were determined and compared to the cellular makeup of 100 colonies formed from marrow. Any difference between colonies from blood and marrow could occur by chance alone 30 to 50 per cent of the time.

<table>
<thead>
<tr>
<th>Source of Stem Cells</th>
<th>RBC-Producing</th>
<th>WBC-Producing</th>
<th>Megakaryocytic</th>
<th>Mixed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood leukocytes</td>
<td>36</td>
<td>24</td>
<td>21</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>Femoral bone marrow</td>
<td>47</td>
<td>22</td>
<td>19</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>

**The Treatment of Radiation Injury by Infusion of Marrow from a Pool of Donors**

S. Vlahovic, V. Vlahovic and J. W. Ferreebe. From the Mary Imogene Bassett Hospital (Affiliated with Columbia University), Cooperstown, N. Y.

In a noninbred population of Swiss-Webster mice, a study has been made of survival rates following lethal (750r) exposure to radiation. When antibiotics were given, infusions of marrow from a pool of 30 to 60 donors were as effective in inducing survival as infusions of autologous marrow. Survival was less good when the marrow infusions came from a single donor, or from a pool of 15 donors, or from a pool of 120 donors, irrespective of the numbers of cells injected. The observations may be evaluated on the basis of the H-2 heterogenicity existing in the population. Marrow from a single donor or a pool of too few donors is unlikely to provide a compatible donor when hetero-
ADMINISTRATION OF POOLED ALLOGENEIC BONE MARROW TO LETHALLY-IRRADIATED RATS

William H. Knospe, Johannes Blom and Minnie H. Davis. From the Walter Reed Army Institute of Research, Washington, D. C.

These studies were designed to evaluate the effect of pooled allogeneic bone marrow upon the course of the secondary syndrome. Sixty female Wistar rats were randomized into four groups of 15 animals each. Group A received no treatment. Groups B, C, and D received 1000 r total-body irradiation from a Co60 source. Group B received no marrow. Each animal of group C received (41–102 x 10^6) allogeneic nucleated cells i.p. from a single donor 5 hours postirradiation. The 15 animals of group D were divided into three equal subgroups. Each of these five animals received pooled allogeneic marrow i.p. from the five donor rats (30–60 x 10^6 cells). All animals in this experiment were randomly bred to preclude inbreeding.

No animals in group A died. All animals in group B died by day 15. At 30 days, 14 of 15 animals in group C and nine of 15 animals in group D survived. At 60 days 10 animals in group C and eight animals in group D survived. Average weights of group C and D have been virtually identical throughout the postirradiation period of 9 months to the present time. Average weights of C and D have lagged significantly behind group A throughout the period of observation. None of the animals of group C and group D has demonstrated overt signs of secondary disease.

These results raised the question of a weak or absent histocompatibility barrier in the utilized strain in spite of random breeding. A second experiment was designed utilizing pooled allogeneic marrow derived from individuals of five different strains. Single donor marrow was obtained from Sprague-Dawley rats and pooled marrow was obtained from Sprague-Dawley, Fisher, NIH Black, Long-Evans, and Sherman rat strains. The experimental design was similar to the first experiment except that the number of animals in each group was increased to 20. Marrow was injected into tail veins of Wistar females given 825 r total-body X-irradiation (300 kvp) 5 hours previously.

In the second experiment, all animals of group A survived and all animals in group B died within 16 days. Fourteen animals of group C and 13 animals of group D survived at 7 weeks postirradiation. Up to the time of this report, these two groups have not differed significantly in their average weights but remain below the controls.

These studies suggest the presence of a weak or absent histocompatibility barrier in the rat, making it an undesirable species to study secondary disease. The recently reported studies of Malinin and associates at the last Protection and Recovery Discussion Meeting raise the question as to whether a similar mechanism may not be operating in their experiments utilizing pooled guinea pig huffy coat. Although the effectiveness of allogeneic marrow pooling as a method of modifying secondary disease has been neither established nor negated by our studies, they do emphasize the necessity of rigorously controlled experiments in the study of this problem. Similar experiments utilizing other mammalian species are planned.

SOME OBSERVATIONS ON LONG-TERM STORED DOC BONE MARROW

T. I. Malinin, C. E. Brodine and V. P. Perry. From the Tissue Bank Department, U. S. Naval Medical School, Bethesda, Md.

Approximately 25 ml. of bone marrow was aspirated from each of 10 experimental animals. It was mixed with an equal volume of Morgan's 199 tissue culture media contain-
ing heparin. The suspension was centrifuged and the supernatant fluid used to prepare a 30 per cent solution of dimethyl sulfoxide. The packed cells were then resuspended in an equal volume of this solution, frozen at a rate of 1 C. per minute and stored at −150 C. for 1 year.

At the end of 1 year's storage each dog was given its own marrow 1 day after the exposure to lethal doses of radiation. Out of 10 experimental animals, eight survived beyond the 60-day period.

One ml. of frozen, stored, and thawed bone marrow suspension was left in each storage container, and the present report is based on the study of smears prepared from these aliquots of bone marrow. Atypical changes were found in 3 to 30 per cent of bone marrow cells. These consisted of the loss of nuclei, the condensation of chromatin around the nuclear membrane resulting in the "nuclear vacuolation," and the change of the usual tinctorial properties of the cytoplasm. The remaining cells maintained a usual morphologic appearance. Such atypical cellular changes were not observed in smears prepared from bone marrow stored for 10 days. The severity of these changes showed no correlation with the survival of experimental animals.

**INTENSIVE RADIOTHERAPY OF LYMPHOMA WITH THE AID OF FROZEN AUTOLOGOUS BONE MARROW**

N. B. Kurnick. From the Veterans Administration Hospital, Long Beach, and University of California, Los Angeles, Calif.

It has been observed that patients with stage 1 lymphoma who receive cancerocidal X-ray therapy to the affected nodes rarely have recurrence of disease in the treated area. It appeared likely, therefore, that administration of cancerocidal X-ray therapy to all the node-bearing areas and spleen might markedly improve the results of therapy of human lymphoma. Since such intensive radiotherapy may be expected to produce dangerous bone marrow depression, storage of autologous bone marrow by freezing in glycerol-tissue culture medium was planned as an adjunct to this program. In this way, patients who suffered severe depression of hematopoiesis could be reinfused with their own bone marrow, while those who did not suffer marked depression could be randomly divided into two groups, one of which would receive bone marrow at the end of irradiation therapy, while the other would serve as controls.

All patients with lymphoma who had not received radiotherapy to more than one region or who had not received three or more courses of chemotherapy were included in the study, unless there was evidence of liver or bone marrow involvement. Between September 1957 and September 1959, bone marrow storage was performed on five patients with Hodgkin's Disease, three patients with lymphosarcoma, and one patient with giant follicular lymphoblastoma. All of these were treated in the conventional manner, including low dosage of radiotherapy to affected areas only and chemotherapy. Beginning in September 1959, patients who had been subjected to bone marrow storage were randomly divided into a conventional therapy group and another group which was subjected to X-ray or Co⁶⁰ therapy to the cervical, axillary, supraclavicular, mediastinal, peri-aortic, inguinal, and femoral nodes and spleen. A total of 2400 to 3000 rad mid-sagittal plane dose was given to each of these areas. Therapy was administered sequentially to each area, 400 rad per treatment, administered daily except weekends.

By random selection, five patients with Hodgkin's Disease, three with lymphosarcoma, and one with giant follicular lymphoblastoma were in the intensive radiotherapy group. There were 10 patients with Hodgkin's Disease and two patients with lymphosarcoma in the "conventional therapy group." Because of leukopenia below 1500 cells per mm.³ or thrombocytopenia below 40,000 per mm.³, persistent for more than 3 days, two of the patients with Hodgkin's Disease in the control group were reinfused with bone marrow. Because of the same criteria, two of the patients in the intensive therapy group received bone marrow reinfusion at the conclusion of the therapy. Of the re-
ADOPTIVE IMMUNITY TO AILOCENEIC AND XENOGENEIC SKIN GRAFTS IN THYMECTOMIZED-IRRADIATED, ISOGENIC MARROW-RESTORED MICE

William E. Davis, Jr. and Leonard I. Cole. From the U.S. Naval Radiological Defense Laboratory, Biological and Medical Sciences Division, San Francisco, Calif.

Thymectomy of 10 to 12-week-old mice followed by X-irradiation and restitution with syngeneic bone marrow cells from normal donors results in an impairment of immunologic response to allogeneic skin grafts, but not to xenogeneic grafts. The following experiments were performed to determine whether bone marrow and lymph node cells from thymectomized-irradiated donors are capable of transferring immunity adoptively to thymectomized-irradiated recipients. The donors were adult LAF1 mice, thymectomized and exposed 2-3 weeks later to 880 rad of X-radiation. They were restored with $5 \times 10^7$ normal syngeneic bone marrow cells. Four weeks later, at a time when impaired response to allogeneic skin grafts was still in evidence, these mice were killed and $10^7$ marrow cells (and in some cases $10^7$ node cells) from them were injected i.v. into a second group of irradiated (880 rad) adult LAF1 mice, which in turn had been thymectomized 2 weeks earlier. This second group was then challenged with allogeneic (C3H/HeJ) and xenogeneic (rat) grafts 1 day later. Appropriate sham thymectomized or intact controls were also employed.

The resultant mean survival times (MST in days) for allogeneic skin grafts showed an impaired response in the thymectomized recipients when thymectomized donor marrow (MST > 54) or marrow + node cells (MST > 46), or sham-thymectomized donor marrow cells (MST > 41) were injected. By contrast, when sham-thymectomized donor marrow + node cells were injected, the response was relatively vigorous (MST 14.5). Intact irradiated recipients injected with intact donor marrow or marrow plus node cells showed MST values of 33 and 16 days, respectively, for the allogeneic skin grafts.

The survival times for the xenogeneic grafts followed the same pattern but were, in general, shorter than for the allogeneic grafts. In thymectomized recipients injected with thymectomized donor marrow or marrow + node cells, the MST were 35 and 29 days, respectively, and after injection of sham-thymectomized donor marrow the MST was 43 days. By contrast, infusion of marrow plus node cells from sham-thymectomized donors resulted in earlier rejection time (MST 16). Intact irradiated recipients injected with intact (control) donor marrow, or marrow + node cells, rejected rat skin grafts at 27 and 19 days, respectively.

It is evident from the above data that restitution of immunologic reactivity in bone marrow and lymph nodes of marrow-restored irradiated mice is profoundly influenced by the presence of the thymus. However, as shown by these and other data, the effect on xenogeneic grafts is much less marked than on allogeneic grafts. Indeed, we have observed that where allogeneic host and donor share the H-2 locus, a long-lasting, probably...
permanent, impairment of the response results in thymectomized, irradiated adults. Therefore, it appears that in adult irradiated mice, the thymus is most critically involved in the homograft response to non-H-2 iso-antigenic differences: non-H-2 > H-2 > interspecific.

**Intestinal Helminths in X-Irradiated Mice with Implanted Bone Marrow**

*W. Friedberg, D. N. Faulkner, J. K. Abbott and M. H. Friedberg. From the Civil Aero-medical Research Institute, Federal Aviation Agency, Oklahoma City, Okla.*

In the course of examining sections of gastrointestinal tract from X-irradiated (950 r) mice implanted with rat bone marrow, from irradiated mice implanted with syngeneic marrow, and from normal control mice, we observed that the two groups of irradiated animals had more intestinal helminths than the normal controls. From the number of worm fragments (cestodes plus nematodes) in the tissue sections, the mice with the implanted rat marrow seemed to be the most heavily infected. We saw relatively few intestinal parasites in the normal animals. The tissue samples were taken 23 days after irradiation when the rat-mouse chimeras were undergoing the foreign bone marrow reaction (secondary disease) as indicated by the loss of body weight and atrophy of splenic white pulp. The cestode *Hymenolepis nana* was identified by the presence inside a villus (in a rat-marrow-treated mouse) of a cysticercoid which had a rostellum armed with one circle of 24 to 26 hooks. The nematodes were not identified.

Natural resistance and acquired immunity normally keep the number of intestinal parasites under control. Apparently the marked depression in function of the immune system caused by the X-irradiation permitted a massive helminth infection. Recovery of the immune system after irradiation is more complete in mice treated with syngeneic marrow than in rat-mouse chimeras. This could explain the difference in the number of intestinal helminths between the two groups of irradiated mice. Parasite counts in rat-mouse chimeras by standard methods at several times after irradiation and studies with parasite-free animals would be helpful in determining the role, if any, of intestinal parasite infection in secondary disease.

**Nitrogen Balance in Radiation Chimeras**

*W. H. McArthur. From Knoxville College, Knoxville, Tenn.*

Mice receiving lethal doses of X-irradiation can recover if they are injected with normal bone marrow cells. If the injected cells are genetically different from the irradiated host, the animal survives the radiation syndrome, but may die later because of a secondary disease not related to the acute effects of irradiation. Secondary disease is a consequence of an immunologic reaction between the grafted cells and the host animal. The body weight loss and the failure of hair to grow in foreign marrow chimeras suggest the mice are suffering from a metabolic starvation. Kretchmar and Congdon (Am. J. Physiol., 200, 102, 1961; Exptl. and Molecular Pathol., 2, 277, 1963) have studied the biochemistry of the metabolic disease process, on the assumption that it involves a disturbance in protein metabolism.

Food intake of homologous-marrow treated mice is adequate and comparable to that of normal or isologous-marrow treated animals. We have previously reported results that confirmed the similarity of food intake among normal and isologous or homologous marrow-treated irradiated mice. We have also reported that mice of these three groups had comparable degrees of positive nitrogen balance. Recent experiments show positive nitrogen balance, but no gain in body weight among the homologous chimeras.

Experiments consisting of 24 mice of which eight were normals, eight isologous, and eight homologous covered a period from day 1 through day 90.

Both isologous and homologous marrow-treated animals were in positive nitrogen balance and retained more nitrogen than did
normal control mice for the first 30 days. Weight gain of isologous marrow-treated animals was the same as that of normal mice. Animals given homologous marrow gained little or no weight.

Animals treated with isologous marrow maintain a normal rate of gain in body weight through 90 days, but retain significantly more nitrogen than normal animals. Mice given homologous marrow fail to gain weight, but retain twice as much nitrogen as normal mice. Both marrow-treated groups show the same degree of increased positive nitrogen balance.

Our results indicate that correlation between nitrogen balance and food intake does not change significantly during the 90-day period.

We speculate that nitrogen is directed into some abnormal compartment or compartments in animals with secondary disease, and that this is related in some way to the immunologic conflict between grafted homologous cells and the host animals.

Our results show that diarrhea is not an invariant feature of the metabolic syndrome.

LYSOSOMAL ENZYMES IN TRAUMATIZED TISSUE

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Lysosomal enzymes (RNase, DNase, β-glucuronidase, and β-galactosidase) increased in experimentally inflicted poultry bruises. Activities were highest in the bruise; elevated but slightly less, in the bruise periphery; and normal in symmetrically located control muscle. The trend in activity of all these enzymes in response to injury was similar, but differed in magnitude. Increasing the severity of contusion resulted in a greater maximum activity and an increase in the time post-bruise required to reach this maximum. When chickens were successively bruised (2- to 8-day intervals), the general effect was an earlier detection of accelerated activity and an earlier occurrence of the maximum activity. This was consistent with an acceleration of healing by repeated bruising. Free, lysosomal-bound, and total activities increased in healing bruises. The percentages of bound enzymes in bruised tissue were 86, 79, and 51 for DNase and β-glucuronidase, respectively. Due to their low activities, free and bound activities in normal muscle were not determined. In conclusion, lysosomal enzyme activities did increase in healing poultry bruises and these activities were influenced by severity of bruising and prior bruise history.

BONE MARROW TRANSPLANTATION AFTER INTRAPERITONEAL ADMINISTRATION OF DMBA

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The polycyclic hydrocarbon, 9,10-dimethyl-1,2-dibenzanthrace (DMBA) is a well-known chemical carcinogen in mice. In previous work, attempts to reverse the acute chemical toxicity of DMBA by AET protection and bone-marrow transplantation were made. These experiments are part of a program aimed at investigating the acute pathologic effects of representative members of the major classes of chemical carcinogens and certain pathogenic viruses in order to carry out protection and recovery experiments that might alter the pathologic changes.

In the earlier work, AET protection and bone marrow transplantation did not prevent the mortality resulting from lethal doses of DMBA, although in one instance marrow injection was associated with fast recovery of the damaged hemopoietic system. The present experiments showed in a clear fashion that marrow injection aided hemopoietic recovery from the toxic effects of i.p. administered DMBA even though it did not increase 30-day survival.

Groups of B6D2F1 male or female mice were given i.p. 1.5 or 2.5 mg of DMBA dissolved in olive oil. Three days later half of the animals received 100 x 10⁶ or, in one instance, 40 x 10⁶ isologous bone marrow
(IBM) cells i.v. On the 7th and 14th days after DMBA, mice were killed and autopsied from the control and the bone marrow-treated groups. Animals that survived to days 21 and 28 were also examined in some experiments. Body, spleen, and thymus weights were taken at autopsy, and many tissues removed for histologic study. In three experiments, there was greater histologic recovery of the spleen, bone marrow, thymus, and lymphatic tissues in the marrow-treated group than in the DMBA control. In a fourth experiment, there was no difference. Isologous bone marrow did not increase survival of mice after i.p. DMBA because of the peritonitis produced when this route of administration was used. Pancreatic necrosis was present with the peritonitis. Death from peritonitis rather than hemopoietic failure was the major autopsy finding in all of the DMBA experiments. Further experiments are now being carried out with the oral route of administration of DMBA to try to achieve a hemopoietic death that might be prevented by marrow therapy.
Protection and Recovery Discussion Meeting

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