CLINICAL REPORT

Conference on Clinical Aspects of Radiation Injury and Transplantation of Bone Marrow and Other Organs*

Arranged by C. G. Zubrod and A. Hollaender

MORNING SESSION, SEPTEMBER 18, 1959

DR. JOSEPH SMADEL, Associate Director for Intramural Research, National Institutes of Health, opened the meeting at 9:00 a.m. by extending a welcome from the National Institutes of Health to the members of the conference.

The conference concerned itself with three topics: chemical protection against radiation; radiation injury and organ transplantation; and "germ-free life" for the patient with bone marrow damage.


Dr. David G. Doherty reviewed the chemistry of AET, emphasizing the need for converting the compound to its active form, mercaptoethyl guanidine (MEG) by adjusting the pH of the solution to 7.0 to 7.8 with phosphate buffer. Failure to do so results in rapid and marked loss of activity by conversion to products with lower activity, i.e., 2-amino-thiazoline or bis(guanidoethyl) disulfide.

In order to avoid marked loss of activity of AET, the following conditions are necessary: (1) it must be stored in crystalline state, and kept dry in a desiccator; (2) if given by mouth, should be weighed out just prior to administration, and given by itself on empty stomach; (3) if it is to be used in solution orally, it should be made up fresh and given at once, or given sufficiently buffered with phosphate to keep pH above 7.0. In aqueous solution, there is 47 per cent loss within 20 minutes. New information in mice showed that return of the normal mitotic rate in intestinal tract occurred even after 2000 r in presence of MEG. This was also true of protein synthesis by liver. MEG and mercapto-ethyl-amine (MEA) were compared under identical conditions in mice. The LD50's were 1150 r for MEA and 1450 r for MEG. However, MEA was active for a short time only, presumably because of rapid metabolism.

Dr. Margaret G. Kelly reviewed toxicity of AET, pointing out that it is more toxic for dogs than rodents and does not protect dogs against radiation or nitrogen mustard. Amino-propyl-isothiourea protected mice against radiation but not against nitrogen mustard. Amino-propyl-methyl-isothiourea gave

Submitted Feb. 19, 1960; accepted for publication Mar. 8, 1960.

*Abstract of an exchange of views of interested workers held at the National Institutes of Health, Bethesda, Md., on September 18 and 19, 1959. These conferences are held three times yearly and an abstract of the proceedings published in Blood.

1A listing of the participants in the conference begins on page 1349.
protection against neither. AET did not interfere with tumor-damaging capacity of HN2 or local x-ray but did interfere with antitumor effect of total body radiation. Dr. Joseph E. Schlosser reported on 59 patients given AET and radiation. The toxic effects, when given in solution, were nausea, vomiting, rash, hypotension, sneezing, stomatitis, dyspnea and hemolytic anemia. If the compound was kept under anhydrous conditions and given by mouth, few of these reactions were seen. The effect on systemic effects of radiation were difficult to judge, but no protection of tumor against radiation was noted. Dr. Paul K. Smith reported on pharmacologic disposition of MEG in man after a dose of 8 mg./Kg. by mouth. The half-life of MEG in plasma was 12 to 16 hours. Most of this appeared in urine as MEG and unidentified oxidation products. Sulfates and tyrosamine were also found. Dr. Bruce I. Shneider reported on results of administration of AET to patients. It did not prevent leukopenia following nitrogen mustard. Dr. David B. Jacobus described the extensive screening program of Walter Reed Army Medical Center in a search for new protective chemicals. He also reported results of dog studies in which protection against the acute effects of 1500 r was achieved by a combination of MEA, cysteine, dihydroxy diphenyl and para-amino-propio-phenone (PAPP). Dr. R. R. Oterman reported upon results in dogs and monkeys using PAPP alone or PAPP with AET. The degree of protection from PAPP was shown to be related to extent of resulting methemoglobinemia. Dr. Joseph W. Byron described studies with AET in various ascites tumors treated with intraperitoneal nitrogen mustard. Dr. Willie Smith reported on radiation protection afforded rats, mice, hamsters and guinea pigs with administration of the endotoxin from S. typhosa. Colchicine was also shown to protect if given several days before radiation. The mechanism of protection of both agents was stated to be entirely different from the mechanism of sulfhydryl or methemoglobin protection. Dr. Sadahisa Kawamoto reported that 8-methoxy psoralen, the ultraviolet protective agent, also prevented x-ray leukemogenesis in mice but had no effect upon leukemia due to methylcholanthrene.

Afternoon Session, September 18, 1959

Session II. Transplantation of Bone Marrow and Other Organs. Dr. E. Donahue, Thomas, Presiding

Dr. Georges Mathé reviewed the Yugoslav reactor accident in which six scientists had received 400 to 1000 rem total body radiation and five were subsequently given homologous bone marrow. Recently one of the five survivors had begun to show cataracts. They have had some psychic depression, and there is a question of sterility, but otherwise they are in good health without hematologic disorder or evidence of secondary disease.

Dr. Gould A. Andrews gave a further follow-up on the Y-12 accident. The men complain of lack of well being and muscle weakness. The erythrocytes are normal, and leukocytes and platelets are low normal. Dr. Arthur L. Kretchmar presented the data on aminoaciduria in the Y-12 victims. There was no general increase in total amino acids, but there were marked increases in the
urine of taurine and serine, and appearance of β-amino-isobutyric acid. Kretchmar also gave data on a patient who died 35 hours after the Los Alamos accident. There was no general aminoaciduria, but qualitatively there were bizarre changes in amino acid pattern, with appearance of compounds as yet unidentified. The time course of taurine excretion in the three specimens voided before death was 44 mg. in first 14 hours, 80 mg. from 14 to 17 hours and 91 mg. in 17 to 22 hours. This was a total of 215 mg. as compared to normal values of 20 to 42 mg. in 24 hours.

Captain E. R. King reviewed the plans of the Navy for management of radiation casualties. Thomas summarized the current consensus concerning care of radiation casualties, stressing general medical measures and protection against infection, especially against hospital strains of pathogenic organisms. Transfusion of blood and platelets should not be done routinely, but only for specific indications. Antibiotics probably should not be given prophylactically, but the bacterial flora should be followed by frequent cultures and infections should be treated promptly. The question of bone marrow transplantation need not be answered immediately, and the decision can be made one to two weeks after exposure when all facts are available about dosage of radiation, and the course of the patient demonstrates the need for attempted transplant.

Mathé reported on the treatment of acute leukemia with total body radiation and homologous bone marrow. Twelve patients were treated (with sublethal doses after steroid hormones) while in relapse, and no remissions were obtained. Six other children were treated during remission induced by corticosteroids and maintained on 6-mercaptopurine. They received 800 to 900 rad, then 10 billion cells of homologous marrow 7 to 13 days later. In two patients, there was no bone marrow restoration, and death due to marrow failure and infection occurred (staphylococcal in one, fungal in the other). In the other four children, myeloid restoration occurred. The take of the donor bone marrow was discussed: in two patients who had a long survival, erythrocytic antigens of the donor type were found for three months at a low percentage; in one who died one month after irradiation, the rate of the erythrocytic antigens of the donor type increased until death, and extramedullary myelopoiesis was seen at autopsy. In the fourth, a boy who died 43 days after irradiation and who received bone marrow from his mother, the sex-linked appendixes of the granulocytes were male before bone marrow transfusion and female after transfusion until death. In the other two patients the clinical state was satisfactory after myeloid restoration until the forty-fifth day. At that time, fever, skin infection, cough and digestive symptoms occurred with disorders in gamma globulin level, lymphopenia and lymphoid aplasia of the lymph nodes. This syndrome disappeared after about one month when the red cells of the donor type disappeared. The patients were in excellent health until six months for one and five for the other, when the leukemic process recurred. In the two other patients who received bone marrow from their mother, the clinical states were not ameliorated by myeloid restoration but worsened: erythrodermia, high fever, digestive trouble and lymphopenia occurred, with fungal infection in one, hepatitis and pneumonia in the other.
The clinical, hematologic and histologic (lymph nodes, skin, ...) symptoms of this "secondary syndrome" (appearing after myeloid restoration) were compared with the homologous disease observed in homologous chimera animals.

Andrews reported on 11 patients (12 trials) with acute leukemia treated with whole body radiation, 200 to 900 r in mid-plane, from a cobalt-60 source. Only two patients also received homologous marrow. All patients were in relapse; antimetabolites were discontinued but corticosteroids were maintained. The results were as follows: exacerbation with proliferative phase and death, 2; partial sustained suppression, 3; complete remission but death in aplastic phase, 2; complete remission in 3 patients, only one of whom received marrow; and early death in 2 patients. In comparing leukemic patients with victims of the Y-12 accident, there was noted a marked difference in the time (after radiation) at which the leukocytes, reticulocytes and platelets began to rise. This was 40 days after radiation for the Y-12 accident, and 20 days after radiation for the leukemia patients. The reason for this is not apparent. Andrews concluded that there was no conclusive proof of bone marrow take, and that complete remission of acute leukemia could occur from total body radiation alone.

Thomas, in considering all the data to date on homologous bone marrow transplants, concluded that apparent takes are due to variations in results obtained with the Ashby technic. The hematologic findings after radiation and homologous transplant can all be explained on the basis of regeneration of the patients' own marrow. He reported, in addition, one patient who received 900 r and homologous marrow. Recovery of marrow function occurred after 30 days, but Ashby studies showed that the recovery was due to the patient's own marrow and not to the homologous marrow. This patient demonstrated that autogenous marrow recovery can occur after very large doses of radiation when death is postponed by proper clinical care. The patient died of recurrent leukemia after 150 days. Thomas also summarized the dog data to date indicating that intensive medical care can save dogs given 750 r, although this is an LD100 for dogs cared for in more routine fashion.

Dr. William McFarland reported on his experience with total body radiation of patients with solid tumors given 700 r mid-body dose. He also noted that leukocytes and reticulocytes began to return at 20 days.

Dr. Nathaniel B. Kurnick reported on the use of homologous bone marrow in the treatment of five patients with aplastic anemia. No radiation was given and there were no takes. He also described his current studies with autologous bone marrow. All patients with solid tumors who are to undergo radiation therapy or receive bone marrow depressant drugs have bone marrow collected and stored. Marrow is also stored from all patients with acute leukemia in remission and all patients with lymphoma. Patients with diffuse metastases from testicular cancer are given 1200 r to torso in the mid-sagittal plane. One month after autologous bone marrow there is a sharp upswing in peripheral blood values. In identical situations without bone marrow, the upswing of blood counts does not occur for five months. Similar good results obtained with autologous marrow in the treatment of bone marrow aplasia from 60 mg. of nitrogen mustard. Kurnick is now studying dose of autologous marrow
needed to restore bone marrow. He has been using 3 billion nucleated cells but believes that 400 million cells may be enough. He has also treated two patients suffering from agammaglobulinemia with fetal spleen transplants. In one patient there was no result, and in the other a beta globulin, but no gamma globulin, appeared in the serum.

Dr. John Mannick reported on the successful homologous transplant of a kidney to a nephrectomized dog one month after radiation and successful bone marrow take from the same female dog. The donor kidney was normal functionally and histologically for 73 days, when the dog died from sudden thrombopenia but without other evidence of secondary disease. The bone marrow of recipient dog was hyperplastic at autopsy, and female leukocyte markers persisted in peripheral blood to death.

Dr. John Merrill reported on the experience with kidney homotransplants in man after radiation and homologous marrow. In one patient neither kidney nor marrow took. In another patient, there was evidence of some take of both kidney and marrow from mother. In a third patient who was presumed an identical twin, the preliminary trial with skin showed rejection by recipient. After 250 r followed in one week by 200 r, a successful kidney transplant was made from the now presumed fraternal twin. Six months later, following sunburn, the patient had hematuria and flaking of skin graft. This cleared, but the patient had a similar episode after another sunburn. The Brigham group have had three other experiences with sublethal radiation before homologous kidney transplant: In one patient there were blood type differences and no take; in a second there was take but death from sepsis; in the third, transplant could not be done after radiation.

Dr. Joseph E. Murray reported upon a similar patient whom he had seen in Amboujet's clinic in Paris. A patient in renal failure received a kidney transplanted from a nonidentical twin after sublethal radiation. At eight weeks, renal function was normal. No bone marrow was used.

Dr. Gustave J. Dammin presented the histology of the kidney transplants of Merrill's patients. He noted that (1) the pattern in the kidney was characteristic of homograft rejection; (2) sublethal radiation prevented appearance of rejection pattern; (3) normal lymph node structure was slow to return after whole body radiation.

Oberman stated that in monkeys, death from secondary disease resulted if bone marrow was given immediately after 550 r (LD10). If a homotransplant was given 48 hours after radiation, the monkeys did not die.

Morning Session, September 19, 1959

Session III. "Germ-free life" for the Totally Irradiated Patient. DR. J. W. FERREBEE, PRESIDING.

Ferrebee gave a summary of the problems of the irradiated patient with emphasis on the problem of infection. Sources of infection could be organisms in the environment or those present in the patient's tissues. Subjects for inquiry are ways to clean up the environment, and ways to clean up the patient. The role of infection in secondary disease needs study. So does the problem
of the repopulation of lymphoid tissues, as these tissues play important roles in defense against infection. Perhaps the studies of the irradiation of germ-free animals can provide some of the answers.

Dr. Stanley M. Levenson: When germ-free chicks are radiated, the LD$_{50}$ is 100 r higher than the LD$_{50}$ for normal chicks in White Leghorns, but not in California Greys. Germ-free mice of the Lobund strain showed a delay in onset of death, but all died after 900 to 1800 r. In another study, 40 per cent of germ-free mice given 700 r under germ-free conditions died with deaths beginning on the eleventh day, while other germ-free mice contaminated with E. coli two weeks before 700 r, were all dead by the eleventh day. In applying the lessons of germ-free life to the operating room, the rigid tanks are useless. However, the experience gained with Trexier’s plastic tank seems directly applicable both to the operating room and to the isolation of a patient in his room. Such operating room and isolation room units are now under trial. In the operating room, the wound is completely isolated from the operating room personnel, while in the isolation room the entire patient is kept from all contacts with unsterile things and people. Rapid advances are being made, and such units may be available within next six months.

Dr. Philip Trexier: This subject is only one facet of a bigger problem, the protection of an individual in an exotic environment. Similar problems of isolation of subject arise in certain manufacturing processes, in radioactive isotope work and in space travel. There is no question now that the complete isolation of the subject can be achieved, but the question remains as to what price one is willing to pay in terms of loss of maneuverability.

Dr. Walter L. Newton: While germ-free technic can isolate the individual from his environment, they would not necessarily contribute to knowledge of protection of the patient from his own organisms. It is the latter which presents the greatest problem here. Guinea pigs die rapidly when removed from germ-free existence; mice get diarrhea, but survive. Germ-free animals become more susceptible to nonpathogens such as Trichomonas and to non-specific parasites.

Dr. Helmut Gordon: The rationale for using germ-free animals for radiation research is that one can observe a relatively pure radiation effect. What are the specific characteristics of the germ-free animal? He lacks parasites but also totally lacks response to antigens, both bacterial and nonbacterial. There are differences in serum proteins because of lack of gamma globulins. Although there is lowered resistance to ordinary bacteria, other systems are surprisingly normal. In rodents, there is a larger cecal sac which is not associated with any deleterious effects. When radiation effects are compared in conventional and germ-free rats, at 300 r all survive; at 400 r there is a higher survival rate in germ-free. After 600 r all animals died, but germ-free animals had longer survival.

Dr. George Brecher: The germ-free rats dying late showed severe anemia as the only striking finding.

Ferrebee: In “cleaning-up” a subject with antibiotics one can destroy pathogens, but this allows overgrowth of nonpathogens. What happens when
a conventional animal is placed in a germ-free environment and given antibiotics?

Gordon: If this is done with penicillin in chicks, there is disappearance of Clostridia and S. fecalis. Nutritionally, these animals behave as germ-free animals. It becomes qualitatively similar to the germ-free animal in some of its defensive characteristics (such as the amount of RE element in the intestinal wall).

W. Smith: When streptomycin alone is given to irradiated animals, there results resistant Pseudomonas and resultant high mortality from septicemia.

Mr. Donald L. Snow: With conventional engineering, including a blanket of sterile air around the operating area, ultraviolet light, electrostatic precipitation and elimination of contact infection, hospitals cannot approach germ-free environment. There are many routes of air-borne contamination still available because of hospital construction. The dust-free environment of certain industries, such as beryllium processing, micro-ballbearing and electronic factories, is far superior to that of hospitals. The results in industry are achieved in part by coveralls, including shoe coverings and in part by a pressurized area of assembly.

Dr. Grant Sanger reported on a controlled hospital study of bactericidal substances for treating all clothing, blankets, floors and the entire ward. Some wards were kept as controls during seven weeks of observation. The conclusions were that the use of bactericidal substances was practicable and safe; 85 per cent of bacteria in air were eliminated cheaply and easily. Treatment of hard surfaces was required four times yearly. It was also necessary to control air inflows.

Merrill reported on his experience with controlled environment in managing totally irradiated patients. Patients were kept in an operating room or a specially isolated room. The environment could be well controlled, but the patients' own organisms could not. For example, a patient with a renal homograft died from infection. There were staphylococci in cut-downs, throat and skin wound when admitted. At one week after radiation her temperature rose to 105 C., and she was given chloramphenicol. Two days later she showed Pseudomonas and yeast in throat and died of Pseudomonas septicemia. In the kidney transplant studies it is the practice to use gamma globulin for treatment of infection, but no prophylactic antibiotics are used. Also tried, without notable success, has been the stimulation of host defenses by non-specific means, using lactobacilli.

Mathé reported on infection in five of six leukemia patients receiving radiation. There was one instance each of staphylococci in lung, fungi in lung and spleen, fungi in brain, fungi in bronchus. He has used prophylactic antibiotics, intestinal antiseptics and gamma globulins. Mycostatin was of no avail in treatment of fungal infections.

Thomas has shown that dogs with radiation and homografts all die of hepatitis or distemper infection. "Secondary disease" in dog seems to be explained by these fulminating virus infections.

Herman stated that of 14 patients receiving radiation and homologous mar-
row, 10 died from infection during aplastic phase; two died of infection plus leukemia, and two died of leukemia. Six of the infections were due to E. coli; three were due to staphylococci; and three of the patients also had fungi or Pseudomonas. Most patients had had prophylactic antibiotics.

Dr. Leandro M. Tocantins observed that in irradiated patients there are few complications for the first five days. In the second week complications are largely related to bleeding. In the third week the danger is from infection and/or bleeding. He recommends more liberal use of transfusions from donor of homograft.

Dr. John P. Utz reported on use of amphotericin in fungal infections. He found it useful for histoplasmosis and blastomycosis but not for candidiasis. If antibacterial prophylaxis is attempted in leukemics, there is an increase in gram-negative septicemias and Candida infections. In a controlled study, Mycostatin was of no value in the prophylaxis of fungal infection.

Dr. Neil Wald reviewed the diagnostic value of symptoms and signs of radiation damage in man.

Dr. Herman L. Oliner showed a film illustrating bone marrow collection and manipulation.

PARTICIPANTS AT THE CONFERENCE

Dr. Gould A. Andrews, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.
Dr. George Brecher, The Clinical Center, National Institutes of Health, Bethesda, Md.
Dr. Joseph W. Byron, Radiobiology Laboratory, Churchill Hospital, Oxford, England.
Dr. Gustave J. Dammin, The Peter Bent Brigham Hospital, Boston, Mass.
Dr. David G. Doherty, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.
Dr. J. W. Ferreebe, The Mary Imogene Bassett Hospital, Cooperstown, N. Y.
Dr. Helmut Gordon, University of Notre Dame, South Bend, Ind.
Dr. Alexander Hollaender, Oak Ridge National Laboratory, Oak Ridge, Tenn.
Dr. David B. Jacobus, Walter Reed Medical Center, Washington, D. C.
Dr. Sadahisa Kaucimoto, M. D. Anderson Hospital, Houston, Tex.
Dr. Margaret G. Kelly, The National Cancer Institute, Bethesda, Md.
Captain E. R. King, National Naval Medical Center, Bethesda, Md.
Dr. Arthur L. Ketcham, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.
Dr. Nathaniel B. Kurnick, Veterans Administration Hospital, Long Beach, Calif.
Dr. Stanley M. Leverson, Walter Reed Army Medical Center, Washington D. C.
Dr. John Mannick, Massachusetts General Hospital, Boston, Mass.
Dr. Georges Mathé, Institut de Radium, De l'Université de Paris, Foundation Curie, Paris, France.
Dr. William McFarland, U. S. Naval Medical Center, Bethesda, Md.
Dr. John Merrill, Peter Bent Brigham Hospital, Boston, Mass.
Dr. Joseph E. Murray, Harvard Medical School, Boston, Mass.
Dr. Walter L. Newton, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Md.
Dr. Herman L. Oliner, The New England Center Hospital, Boston, Mass.
Dr. R. R. O'Sullivan, Institute of Clinical Investigation, University of Tennessee, Memphis, Tenn.
Dr. Grant Sanger, Francis Delafield Hospital, New York, N. Y.
Dr. Joseph E. Schlosser, The Charity Hospital of Louisiana, New Orleans, La.
Dr. Bruce I. Shulman, Georgetown Medical Division, D. C. General Hospital, Washington, D. C.
Dr. Paul K. Smith, George Washington University.

Hematopoietic destruction in the spleens of frogs irradiated with 10,000 r is not prevented by keeping them at low temperature. The fact that their death is delayed is closely associated with maintenance of the elementary function of the hematopoietic tissue: that is, the transformation of reticular cells into blast cells and/or preservation of the cytologic characteristics of blast cell immaturity. Generally, these events are closely related to the presence of cell debris which, in this case, is supplied by the secondary dissolution of the newly formed blast cells.—O. P. J.


Spleens of patients with myeloid metaplasia who had high leukocyte-alkaline phosphatase levels were compared with the spleens of patients whose levels of this enzyme were low normal or lower than normal. Spleens of the patients belonging to the second group showed greater cytologic immaturity, especially in the granulocytic series; there was also greater distortion of the splenic architecture, with almost complete disappearance of lymph follicles, and a striking increase of the reticulum fibers and of fibrous tissue. From the morphologic-cytochemic point of view, one of them was very similar to the spleens of chronic granulocytic leukemia. However the clinical and hematologic picture was that of myeloid metaplasia with myelofibrosis and not of chronic granulocytic leukemia. It is possible that a similar or almost identical pathologic picture may be present in two different but related processes.—W. J. M.
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