CLINICAL REPORT

Summary of Proceedings of the Conference on Chemical Protection and Bone Marrow Transplantation

Held in Oak Ridge, Tennessee, January 8 and 9, 1969

Co-Chairmen: Alexander Hollaender, D. G. Doherty, T. Makino Dan and C. C. Congdon

Chemical Protection of Total Body Irradiation

New Chemical Developments

Doherty (Oak Ridge) reported that nearly 100 new chemical compounds had been synthesized and tested for their protective action. Of the new compounds that showed protective action in lethally irradiated mice, none was better than AET. The new protective compounds, like those previously tested, showed two levels of activity. Some raised the LD₉₀/30-day value from about 700 r in unprotected mice to around 1100 r and others raised it to the AET level of 1400-1500 r. Jacobus (Washington, D. C.) described the present status of the new chemical protection program at the Walter Reed Army Medical Center. Contracts have been made with university and industrial laboratories to produce new compounds of the mercaptan family. About 150 compounds were received and tested, and about half showed some protective activity. Tests were generally made using irradiated mice, but there were additional studies made on irradiated dogs. Plans are also under way to study the possibility of the protective action on the mucous membrane in irradiated humans.

Biological Studies in Animals

Spotnitz (Washington, D. C.) pointed out that although serotonin gave protection against radiation injury in mice and rats, no significant effect was seen in irradiated dogs. Nor was a combination of serotonin and mercaptans effective in dogs. The amount of serotonin that could be administered to dogs was much less than could be administered to mice. Maisin (Washington, D. C.) described autoradiographic studies with tritium-labeled AET in normal mouse tissues and in tumors. There was diffuse labeling of tissue within 5 minutes after administration. Labeled material was also seen in the cell membrane and in mitotic figures. Tumor tissue labeled equally as well as corresponding normal tissue. The problem of proving that the label actually represented the AET in situ is still open. Maisin also reported many studies of mitotic activity in the small intestines of irradiated mice and compared the protective effects of different compounds. These included AET, MEA, APMT, 2-ABT, and serotonin. He also studied treatment with a combination of serotonin and AET, using mitotic activity in the small intestine as a criterion. In general, AET gave the best protective activity when judged by this criterion. Doherty (Oak Ridge) used a bone marrow cell-counting system as well as survival
in irradiated mice to show that the undesirable products in the transguanyla-
tion reaction were not good protective agents. In particular, disulfide com-
pounds were not as effective as the rearranged AET. DiStefano (Rochester, N. Y.) reviewed the pharmacological effect of AET on the small intestine in
different species and of many substituted compounds on the cat. The curare-
like effect of AET and its acetylcholine-like action were discussed. Anderson
(Austin, Texas) described the protective action of AET combined with cysti-
teine on irradiated Rhesus monkeys. In these animals, the LD$_\text{50}$ is about
625 r, but, with protection, some 30 day survival was seen after exposures
as high as 900 r. MacCardle (Bethesda, Md.) found that AET in normal mice
caused rearrangement of the mitochondrial pattern in the liver and the kidney.
The differences normally present between one portion of the liver lobule and
another and between one part of the nephron and another were lost 24 hours
after injection. Later on, normal differentiation reappeared. In microin-
cineration studies, the AET injection was associated with changes in the
kidney ash pattern. Loss of calcium and magnesium with increase in potassium
and sodium was seen. E. E. Schwartz (Philadelphia), who tested the rearrange-
ment products of AET, found MEG superior to the other products from the
standpoints of radiation protection and less toxicity. He also studied intestinal
absorption in protected, irradiated mice. Vogel (Argonne) compared the
protective effects of AET on gamma and neutron irradiated mice. AET af-
forded some protection against injury from both types of irradiation, but was
more effective against injury by gamma rays.

Clinical Research

Schlosser (New Orleans) reviewed his toxicity and radiation protection
studies in humans given AET orally before widefield radiation therapy. He
found that most people can take 1 Gm. of AET orally with minimal toxic
effects. Sometimes nausea and vomiting were seen. He reviewed many case
histories in which AET was used. Morris (Oak Ridge) demonstrated a model
of a new total-body irradiation facility for human beings capable of giving
dose rates as high as 450 r/hour, which has been built at the Oak Ridge
Institute of Nuclear Studies, Medical Division.

Bone Marrow Session

Immune Mechanism

Makino (Oak Ridge) pointed out that the immune response in tissue
transplantation could be approached in terms of host factors, donor cell factors,
and immunogenetic considerations. Gengozian (Oak Ridge) reviewed work
on radiation damage to the mechanism that recognizes a close or a distant
antigen. After irradiation, recovery is more rapid in animals given the more
distant antigen. In very young mice there is a greater antibody response to
the more distant sheep red blood cell antigens than to the more closely re-
lated rat red blood cell antigens. Extrapolating the recognition mechanism
to foreign bone marrow transplantation, Gengozian described experiments
showing that a much higher radiation dose was required for 3 day persistence.
of rabbit bence marrow than for persistence of either rat or hamster marrow in mice. Dameshek (Boston) reviewed work done on rabbits at the New England Center Hospital with 6-mercaptopurine with which it was possible to induce temporary tolerance to human serum albumin. Tolerance to skin homografts was not seen, but survival of the grafts was prolonged with high doses of 6-mercaptopurine. He also mentioned that they were testing 6-mercaptopurine in human autoimmune disorders. Goodman (Oak Ridge) pointed out that several experiments with peripheral blood indicate that an immunologically competent cell is present. The cell is one of the white blood cell series. In her experiments, she added homologous blood to homologous marrow and gave it to lethally irradiated mice. All died within 30 days, whereas if only homologous marrow was used, about 50 per cent were alive at 30 days. She also showed that isologous blood prevents the therapeutic action of homologous bone marrow in lethally irradiated mice, confirming L. J. Cole's report. Goodman thought these experiments might explain some of the difficulties encountered in human bone marrow transplantation as well as large-animal experiments where blood contamination of the marrow is hard to avoid. Congdon (Oak Ridge) commented that histologic studies of mice treated with homologous bone marrow and homologous blood showed an accelerated “secondary disease” picture. The treated mice had successful marrow transplantation, but death occurred in spite of this. In treatment with homologous bone marrow plus isologous blood there was a temporary take of the foreign marrow but the transplant was finally rejected and the animals died from the acute radiation syndrome. Uphoff (Bethesda, Md.) mentioned that she was studying the effect of the H₂ locus on the midlethal dose experiment. In this experiment, the foreign marrow treatment has an unfavorable effect at certain radiation exposure levels. Popp (Oak Ridge) studied requirements for bone marrow cell losses in irradiated mice (F₁-parent combinations), and extrapolated from his figures to obtain the dose requirements of human bone marrow cells necessary for successful transplantation. He estimated that 1.05 x 10¹¹ bone marrow cells would be necessary for the average human. Berrian (Bethesda, Md.) described experiments with mice differing by single genes at the H-2 and H-3 loci. Apparently tissue differences exist when the H-3 genes differ, whereas, at the H-2 locus, cell for cell the tissues are equivalent in antigenicity to skin homografts. There may be a difference between the way antigens from skin homografts are recognized from other types of antigens. At the H-3 locus differences, spleen cells induce tolerance but not immunity. Cohen (Rijswijk, Holland) reported that at lethal radiation exposures in mice the effects of a 24 hour delay in bone marrow injection were no different from those observed with immediate injection on 30 day mortality, secondary disease, and cell dose requirements. These experiments were carried out with isologous, homologous, and heterologous combinations. At midlethal exposure, however, a delay of 24 hours prevented the killing effect of foreign marrow and allowed transplantation. He also mentioned that sensitized female isologous mice did not reject male bone marrow after irradiation as measured by the mortality response. Russell (Bar Harbor, Me.) reported...
that in her experiments on host mice with hereditary anemia, transplantation of isologous fetal liver blood-forming cells occurred without irradiation, although more slowly. Across the H-2 barrier there were no takes; low exposure doses did not permit transplantation. The anemic mice were extremely sensitive to radiation. In genetic combinations that were H-2 compatible but showed slow skin graft rejection, she was able to cure the anemia by transplantation of fetal liver. After the anemia was cured, skin grafts were tolerated and not rejected. Russell feels that more information on histocompatibility genes in man might allow successful tissue transplantation. Owen (Pasadena, Calif.) and Anderson (Austin, Texas) have developed a blood-typing system for the Rhesus monkey that will allow the use of red blood cell markers in experiments on transplantation of monkey bone marrow.

**Transplantation Antigens**

Congdon (Oak Ridge) described the assay system for studying the chemical nature of the transplantation antigens. Most previous work had been based on a secondary response to skin grafting or bone marrow transplantation where the unknown chemical extract was given as a primary injection. Another test system being studied is the primary histologic response in the spleen of irradiated mice receiving chemical extracts. This response is believed to be an immune reaction. Wust (Oak Ridge) reviewed the chemical problems in this work. Using the histologic assay, he thought that a family of substances, protein-polysaccharide complexes, were involved. Activity was found in mucoprotein from human saliva, rat urine, red blood cell stromata, and kidneys as well as other tissues. Berrian (Bethesda, Md.) pointed out the differences between transplantation involving homologous and heterologous systems and thought that they might have a bearing on the chemical isolation problem.

**Experiments with Large Animals**

Cohen (Rijswijk, Holland) described the work with autologous and homologous marrow transplantation in Rhesus monkeys being done at Rijswijk. Female donor marrow given irradiated male recipients was used to demonstrate transplantation of granulocytic elements. Autologous marrow worked well, but even though homologous marrow transplanted, all the monkeys died within a month. It was suggested that whole-blood contamination of the donor marrow might have occurred.

**Sterilizing-dose Values for Tumor Cells**

Bender (Oak Ridge) reviewed the basic studies on killing curves for cells in general, including bacteria and tissue culture work. Most of the studies are on radiation effects, but he pointed out that similar work could be done with chemicals. Miller (New York City) described the work of J. H. Buchenal and his group at The Sloan-Kettering Institute for Medical Research to determine the sterilizing-dose values for cancer chemotherapy agents. The technique is to give graded doses of a drug or radiation to the tumor-bearing animal without regard to the toxicity problem. The tumor is then removed and assayed by transplantation into normal mice. The dose required to prevent
transplantation is considered the sterilizing dose. It is then compared with the LD50-toxicity value of the drug for the animal. In general, alkylating agents have sterilizing-dose values two to three times as great as the LD50 toxicity value. They were not able to determine the sterilizing-dose values for the antimetabolites since no dose tested sterilized. Miller reported that a new compound, Cytotoxan, was unusual in that the LD50-toxicity value and the sterilizing-dose value were about equal. Congdon (Oak Ridge) pointed out that it would be desirable to have the sterilizing-dose values for tumors for the entire spectrum of cancerolytic agents, including all the general protoplasmic poisons. Simmons (Argonne) discussed the work he had done on the radiation doses necessary to prevent the transplantation of tumors. He noted in some of his experiments that small radiation doses to the host used in the assay made the sterilizing dose to the tumor much higher. This kind of work on sterilizing-dose values, whether determined in vivo or in vitro, points to the major problem of clinical application of bone marrow transplantation in irradiated human leukemias. If the marrow transplantation is successful, can the radiation dose or the chemotherapy dose be raised high enough to sterilize all tumor cells in the individual? The basic assumption is that the tumor population is a closed system with no new cells being added from nontumor tissue. Ferrebee (Cooperstown, N. Y.) discussed a possible difficulty with this assumption since a viral etiologic agent might infect the transplanted cells and make them leukemic. Uphoff (Bethesda, Md.) said she was doing experiments in mice that might help settle the virus question. Dameshek (Boston) also pointed out that he considered leukemia to be a disease of an entire system of proliferative leukocytes and not necessarily that of a limited cell population. He thought it would be necessary to control the basic disease process to get a true cure rather than just to obtain a palliative result. Tocantins (Philadelphia) pointed out that, in the virus transfer, a cell population of donor type might not be susceptible to the virus. The importance of using and extending the basic information on the killing characteristics of bacterial and tissue culture cell populations caused by different kinds of agents, as well as the sterilizing-dose values for tumor cell populations, lies in the promise of being able to decide whether the assumption of a closed system in tumor cell populations is correct or not. From the point of view, as well as for other reasons, more work of this type is essential.

"Secondary Disease" as a Metabolic Problem

The “metabolic starvation” in irradiated animals treated with foreign blood-forming tissues has received relatively little study. Friedberg (Oak Ridge) described experiments with labeled proteins in heterologous bone marrow chimeras. The chimeras showed a greater fractional rate of loss of the labeled protein than did normal mice. The protein was not lost by the kidney. He planned to study loss by way of the intestinal tract as a further step. Royal (Greensboro, N. C.) reported on the serotonin content of certain tissues of rat bone marrow chimeras at a time when the “secondary disease” was being established. She found differences between chimeras and mice subjected to isologous bone marrow treatment. There was some evidence that serotonin
plays a role in immunologic reactions of the anaphylactic type, and this led to
the studies in foreign bone marrow chimeras. Royal also pointed out that
some of the very early deaths in mice injected with rat bone marrow, rather
than being exclusively embolic deaths as is usually assumed, might be caused
by serotonin in the bone marrow. She could not detect serotonin in fetal mouse
liver.

Clinical Studies in Man

Retan (Boston) gave the long term follow-up on kidney transplantation in
man performed in the Peter Bent Brigham Hospital. A very high incidence of
recurrence of nephritis was observed in patients with a history of this disease
and who had been treated by transplantation of a kidney from an identical
twin. Kretchman (Oak Ridge) described cases in which HN₂ was given before
autologous bone marrow transplantation. He found that peripheral blood ele-
ments recovered as fast in control cases as in those given autologous marrow.
Andrews (Oak Ridge) and Dameshek (Boston) discussed the toxicity problem
when large doses of HN₂ were used. Intestinal and brain damages were the
problems that limited the use of large amounts of HN₂.

Kurnick (Long Beach, Calif.) thought that autologous bone marrow trans-
plants might be easily covered up by regeneration of chemically injured mar-
row in the HN₂ studies and that the control groups might not show the differ-
ence when the dose was not very great. His group has developed a technique
for procurement and preservation of human autologous marrow in frozen
glycerol. In cases receiving a prolonged course of radiation therapy, they found
that autologous marrow infusion on completion of the therapy returned the
peripheral blood values to near normal levels in about 3 weeks. Marrow in
situ in non-irradiated sites did not readily recolonize the irradiated marrow
sites, whereas infused marrow did.

Bender (Oak Ridge) and Kurnick discussed thawing rates for frozen mar-
row. In mouse marrow, fast thawing gave the best survival, according to
Bender, but Kurnick thought there was some evidence in their work in man
for the superiority of a slow thawing. Since thawing rates like freezing rates
of marrow are critical factors in preservation, this point needs to be settled.

SUMMARY OF PROCEEDINGS OF THE CONFERENCE ON
TRANSPLANTATION OF BONE MARROW

Held in Chicago, Illinois, April 11, 1960

Chairman: L. M. Tocantins

Experimental Work on Rodents and Other Small Animals

Cole (San Francisco) discussed recent tolerance studies in which late (1
year old) homologous mouse chimeras accepted both donor and recipient
type lung grafts while simultaneously rejecting heterologous ones. He con-
cludes that these chimeras have attained a state of "mutual compatibility"
with respect to homologous tissues but are still immunologically reactive to heterologous transplants. Santos (Baltimore) described heterologous (rat-mouse) chimeras given subcutaneous implants of rat and mouse lung tissue at 14–30 days postirradiation or 55–270 days postirradiation. Grafs were examined 35–40 days postimplantation. Rat lung grew best in the first group; however, rat and mouse lung grew equally well in animals of the second group which were apparently true chimeras and had reached a stage of compatibility.

Weiss (Philadelphia) reported that bone marrow from mice with homologous disease was transplanted into irradiated mice of the original donor type. Skin grafts of the original recipient type given 30 days later were rejected at the same time as those on the isologous controls. There was no evidence of tissue antibodies produced by the foreign marrow against the host tissue. Stoloff (Philadelphia) had similar results on circulating antibodies produced by marrow grafts.

Russell (Bar Harbor, Me.) reported on two strains of H2 compatible mice, one with anemia and the other normal, differing so slightly in their genetic composition that skin grafts from one to the other had a prolonged delay in graft rejection time. Fetal liver of the normal strain “took” in the anemic strain without irradiation or chemotherapy and produced a permanently normal blood picture in the anemic mice.

Jacobson (Chicago) reported that mice with a single Peyer’s Patch shielded were irradiated (900 r) and received heterologous or homologous marrow. All grafts were rejected and all animals died. Other mice similarly treated, except that a comparable segment (1 cm.) of small intestine without Peyer’s Patch was shielded, rejected the heterologous marrow and died but only 50 per cent of the homologous group died. Heterologous and homologous controls both survived. There apparently was recovery of the lymphatic tissue in the shielded Peyer’s Patches.

Gengozian (Oak Ridge) described the immune response among mice treated with homologous fetal hematopoietic tissue as comparable to mice treated with adult isologous bone marrow and significantly greater than that obtained in mice treated with adult homologous bone marrow. The response of the former two groups, however, appears to be significantly less than the normal control group of mice, suggesting that transplantation of a compatible graft in the recipient does not necessarily connote a normal physiologic status. Gengozian commented on the number of cells necessary for a successful graft and suggested 15 to 60 x 10⁶ for fetal material.

Shaw (Madison, Wis.) stated that in heterologous chimeras, produced by protecting pigeons which had received 2500 rads (approximately LD 100/30) with ring dove marrow, there is a rapid appearance (4 to 6 days) of host-species-specific hemagglutinins sufficiently potent to produce a high degree of “in vivo agglutination”. Serum samples (day 5 to 14) show antipigeon hemagglutinin titers ranging from 8 to 512. Day 14 sera, using the micro-gel-diffusion technique indicated that there is a host-species-specific precipitin (i.e., chimera serum precipitates normal host (pigeon) serum, including a sample from host animal on day 0, but gave no visible precipitation with
normal donor sera or that of the particular donor]. The most plausible interpretation is that these precipitins were elaborated by the cells of the hetero-transplant.

Santos (Baltimore) emphasized the importance of animal care following total body irradiation including isolation, antibiotics and other palliative measures to decrease mortality. Congdon (Oak Ridge) reported that the blood contamination work of Dr. Goodman suggests that there is a cell in the peripheral blood with immunological potential. Death results from only 0.1 ml. of homologous blood given in addition to bone marrow protection. He suggested that this should be given consideration in the human transplantation work in which aspirated homologous marrow is used or blood transfusions are given post-transplantation.

Cole (San Francisco) reported that giving blood from a different strain of mice along with homologous bone marrow caused the recipient to reject the bone marrow. Sublethal x-rays plus parent strain blood caused a profound anemia in F1 mice with a hemoglobin decrease to 5 per cent and death.

Dorencamp (New York City) said that Cytoxan is lethal for mice using a dose of 150 mg./Kg. on four separate days. Isologous bone marrow in doses of 12.5 to 100 x 10^6 did not protect, even when given within 6 hours after the drug. There may be some slight protection in the larger cell dose levels, i.e., 50 x 10^6 and above. Similar results were reported by Trentin (Houston).

Upton (Oak Ridge) inquired regarding the histological changes of the Cytoxan treated, marrow protected mice. during their short survival. No such evidence is available.

Kretchmar (Oak Ridge) found that the liver undergoes an increase in weight in x-irradiated mice that have been treated with bone marrow cells. This increase is greatest at 12 days after irradiation and is greater in mice that were given homologous cells. Glutamine and glutathione concentration in the livers of these animals is reduced along with a large increase in $\beta$-alanine and $\beta$-aminoisobutyric acid. These chemical changes are also greater in mice given homologous cells. The results are strikingly similar to changes that have been found in livers of tumor-bearing animals.

Experiments on Dogs

Thomas (Cooperstown, N. Y.) reported that out of 35 dogs given 600 to 1700 r and protected with autologous marrow 15 are still in good health and are apparently normal at 12 to 15 months after irradiation except for greying of hair. Hager (Cooperstown, N. Y.) discussed problems in treating irradiated dogs. At first their group gave 1800 r at the rate of 18 r/min. to dogs receiving homologous bone marrow and all of the dogs died. Now they give 1800 at 5 r/min with much better results and less illness. Postirradiation care includes: antibiotics, fluids, hyperimmune sera, injections for hepatitis, distemper, coccidiosis, etc. Hemorrhagic pneumonia is a serious problem. Usually the cell dose is 5 to 10 x 10^6 but one dog received only 100,000 cells and survived. There are now two long term survivors, one a male which still has circulating female leukocytes 15 months postirradiation. The percentage of "takes" has been on the order of 80 per cent. Zukoski (Richmond, Va.) reported that
four out of 14 splenectomized dogs given 600 r and receiving intravenous fetal liver and spleen infusion survived. One died on day 62 undergoing anesthesia but the others are living at 14 to 23 months postirradiation. Only one male was given female tissue and in this animal the female leukocytes in the peripheral blood were evident up to 8 months but had disappeared by the twelfth month. Attempts to protect dogs given 1000 r to 1500 r with fetal tissue infusions have been unsuccessful thus far. Cole (San Francisco) described using lethal irradiation (900 r) plus pretreatment with 6-mercapto-purine (25 mg/Kg.) and protection with multiple injections of homologous marrow from several different donors. One out of five dogs was maintained through the end of the first month. This male dog received 3.5 to 5.5 million female cells on the fifth day. However, during the 4th week there was a precipitous drop in peripheral lymphocytes and the animal succumbed presumably to the syndrome analogous to “secondary disease” in mice. Peripheral blood counts, marrow biopsies and clinical condition indicated definite marrow “takes”.

Identification of the Effective Cell

Billen (Houston) said that in attempts at preserving and identifying the effective cells by the tissue culture method, cell lines have been obtained from bone marrow of C3H mice. Injection of such cells into lethally irradiated isologous mice had no beneficial effect. The use of analogues to prevent maturation of the effective blast cells is being investigated. Early results with 5-bromodeoxyuridine indicate that incorporation of this analogue prolongs the maintenance of an effective cell species.

Preservation and Viability

McGovern (Boston) discussed in vitro studies on viability using a combination of heparin and TC 199. ACD, EDTA and NaCl were also tested. There was a 25 per cent reduction in total nucleated count and phagocytic activity occurring after ten days of storage at 4 C. and a 75 per cent reduction occurs after three months of storage at -70 C. The eosin uptake test correlates well with phagocytic activity at 4 C. but not at -70 C. Thymidine uptake and phagocytic activity correlate well at -70 C. At 4 C., ACD is the best anticoagulant for preserving total nucleated count and phagocytic activity. Heparin and EDTA are less effective in that order. None of the anticoagulants seem to adversely affect thymidine uptake. The importance of studying several parameters of viability is stressed.

Rinfret (Tonawanda, N. J.) reported that they have begun to examine the thermal characteristics of marrow suspensions. A knowledge of such factors as thermal diffusivity, specific heat, latent heat and the manner in which protective additives affect these properties, will be useful in arriving at standard preservation procedures. No equipment suitable for a marrow preservation center has as yet been developed but collaboration with investigators to whom this is a matter of fundamental interest is under way.

Bloom (Buffalo) described a single human marrow specimen, treated in several ways and examined on stained smears before freezing and after
thawing. Additives used included: glucose alone, lactose glucose, glycerol and PVP. Two freezing rates were used: 0.3 C./sec. from 0 to -50 C. in liquid nitrogen vapor and 3 -15 C./sec. from 0 to -50 C. in liquid nitrogen. Storage was over liquid nitrogen at -170 C. All samples were thawed in 37 C. water bath at approximately 0.8 C./sec. for the range of -59 C. to 0 C. Photomicrographs of the cells showed good preservation of form and staining.

Smith (Oak Ridge) is doing long term studies on preserved marrow. One specimen preserved in 15 per cent glycerol and Tyrode's with a temperature drop of 1 C./min. down to -15 C. and then faster the rest of the way to -196 C. showed a survival of $1 \times 10^9$ cells after 1 year. -196 C. is preferable temperature for storage. Rinfret (Tonawanda, N. Y.) commented that cell damage depends on the thermal characteristics of the bone marrow. At -100 C. to -130 C. recrystalization can take place. Therefore, below -130 C. seems to be necessary for preservation.

CLINICAL APPLICATIONS

McGovern described several cases in which autologous marrow transplants were done following HN2 therapy. The patients were used as their own controls.

Thomas (Cooperstown, N. Y.) treated two more acute leukemic patients each of which had an apparently normal identical twin to use as a donor. This makes four such cases in all. In the earlier cases, one received 850 r and had a recurrence two months later, was given 6-MP but died about 15 months postirradiation. The second patient received 1140 r and had a recurrence three months later and died. In the more recent cases, one a 26 year old male was given 1600 r (0.5 r/min.) and fresh marrow from a twin. He made a satisfactory recovery and left the hospital about 40 days later. He had very few irradiation symptoms; however, leukemia recurred in two months and the patient died in three months. The other patient was a 38 year old male with monocytic leukemia who received 2000 r (2 r/min.). He had a very severe G. I. syndrome with diarrhea. About the fourteenth day his white cell count increased and he seemed to be recovering. He then developed progressive jaundice and died on the twentieth day. At autopsy the bone marrow was adequate and there was no leukemia.

Atkinson (Philadelphia) described two cases involving two sets of identical twins. The first one a 2 year old girl with acute leukemia received 250 r and $2.65 \times 10^9$ aspirated marrow cells from her identical twin. There was evidence of a "take" with leukemia recurring in eight weeks. The second one was a 4 year old male with acute leukemia who received 505 r and $2175 \times 10^9$ marrow cells aspirated from identical twin. There was evidence of a "take" but the leukemia recurred 16 weeks later. One year later the donor (the other twin) became leukemic. When identical twins are involved, it is possible that we may be transplanting leukemic rather than normal cells.

Trentin (Houston) reported on a patient with aplastic anemia, believed to be drug induced, in one of two identical twins, age 3. Four and six months after diagnosis, $3.6 \times 10^9$ and $2.8 \times 10^9$ total nucleated cells respectively were transfused from the posterior iliac crests of the well twin to the sick twin
(largely intravenous, partly into the posterior iliac crest). The only change noted over a period of five months after the first marrow transfusion was a possible decrease in the rate of fall of hemoglobin to levels requiring blood transfusion. *Tocantins* (Philadelphia) suggested that *hypoplastic* might be a more fitting term to describe this patient's anemia.

*Atkinson* (Philadelphia) reported evidence of bone marrow "take" in 2 patients injected with homologous aspirated marrow, both involving female donors: five cases with failure, one involving a female donor. Of other patients treated, two acute leukemias and one disseminated neuroblastoma received x-ray (300 r to 800 r), one myelofibrosis (splenectomy one year after transplantation) received Myleran 50 mg. Three aplastic anemia patients and another myelofibrosis patient received prednisolone, in addition to injections of homologous marrow aspirated from normal donors.

*Thomas* (Cooperstown) commented that the term "take" should be more clearly defined and used with greater care. *Kretchmar* (Oak Ridge) stated that remissions were obtained in leukemics by using 300 r to 400 r without bone marrow injections.

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**ERYTHROCYTIC ENZYMES IN ACUTE, EXPERIMENTAL INTOXICATION WITH CARBON MONOXIDE. BEHAVIOR OF CARBONIC ANHYDRASE, OF CATALASE AND OF ALKALINE PHOSPHATASE.** A. Guarino, E. Biondi, F. Boccalatt and A. Mungo. From the Università, Napoli, Italy. Folia Medica 42:376-386, 1959.

Twenty rabbits were intoxicated with carbon monoxide at a concentration of 0.5 per cent. No modifications of the phosphatases were observed; slight diminutions of the catalase and marked diminutions of the carbonic anhydrase (about 36-37 per cent) could be detected. It is suggested that the inhibition of the O₂ transportation cannot explain the carbon monoxide poisoning, and that enzymatic alterations should be also considered in this respect.—*F. d. N.*


M. Bessis and J. Breton showed in a previous work that one can find, in organic material, ferritin crystals morphologically similar to those chemically obtained (C.R.Acad.Sc. 245:1, 271, 1957). They have observed in certain diseases other iron-containing crystals in reticular and hepatic cells: there are empty spaces in the long rows of ferritin molecules. The authors suspected that this is caused by apoferritin mixed in with the ferritin. They observe that apoferritin partially saturated with iron, prepared chemically, shows the same picture.—*G. M.*

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