

Friday afternoon: Problems for future study

Discussion leader: C. C. Congdon

- 1. Effects of whole body irradiation in man and other mammals and their modification by experimental and therapeutic means

J. F. Loutit

This was a carefully thought out and balanced presentation of the present status of the whole problem of radiation accidents and their management. It was pointed out that the protective agents which must be given in advance are likely to be of very limited practical usefulness. After an accident has occurred, the initial problem is of course to establish the dose. For very low doses, in the subclinical level, the health physicist may be in the best position to do this, but with higher doses, the clinician is then most qualified to determine the extent of the injury. Dr. Loutit says that he doubts that the human is so variable in response to radiation as compared with other mammals, as is often stated. He believes that the apparent radiation is due to the different types of radiation and the problems of dosimetry in the various accidents that have occurred. He believes that the dose should be stated in the rad unit, but that the distribution, degree of homogeneity, and duration of exposure are also important. He spoke of the various biological evidences of radiation injury such as fall in lymphocytes, changes in amino acid and creatine excretion, and the time required for development of various clinical manifestations. He seemed rather pessimistic about the specificity and reliability of the various laboratory tests for indicating the severity of radiation exposure. It is his estimate that about 1000 rads would be required to produce completely irrecoverable destruction of the hematopoietic and immunogenetic tissues. In such a circumstance, if a graft could be achieved it would probably be permanent. At lower doses, any graft achieved is likely to be temporary. He pointed out that the harmful effect of homologous marrow seen in some animal experiments at certain sublethal doses is not a universal finding and perhaps has been exaggerated.

Following this there was a rather general discussion on radiation injury and therapy.

Summary of the Important Points in the Conference

1) It appears that patients can tolerate larger doses of radiation than was previously believed. This is possible because of improvements of therapy, including supportive treatment, use of antibiotics, prevention of infection, and use of bone marrow. The fact that these survivals have been seen after very high doses alters somewhat the prognostic implications of certain clinical and laboratory tests. This does not mean that the clinical and laboratory tests were misleading in indicating the dose received, but simply that the lethal range has been pushed up by therapy. 2) The development of effective means of preventing exogenous infection appears to be a significant advance in the therapy of radiation injury, which may be applicable to various accidental and therapeutic situations. 3) Successful homologous kidney transplants have been achieved in patients with some uniformity with the use of radiation to depress the immune mechanism but without any attempt to transplant marrow. There is continued emphasis on the immunologic aspects of these problems. 4) Secondary disease continues to be an area of major interest. In the monkey given homologous marrow, secondary disease appears very rapidly and is a very serious problem. The importance of possible viral infections and of liver involvement in the secondary syndrome were emphasized.

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no need to look for a different type of effect. Dr. Killmann said that he believed that genetic disturbances might require some cell generations to become manifest and thus lead to delay in the maximal hypoplasia.

Monday afternoon: Studies on acute radiation syndrome in man and its treatment after radiation accidents

Discussion leader: K. Williams

re Ges  
↓  
BAIBA  
Tanner  
Creatine  
①

- 1. Problems of diagnosing radiation injury L. H. Hempelmann

Dr. Hempelmann believes that a high urinary creatine excretion is a good index of radiation exposure and that the difference from normal is quite pronounced. (There is a problem of interlaboratory technical differences here, and up to the present it has not been possible for most laboratories to verify these findings on creatine.) He believes that the abnormal creatine excretion can be tied in with the muscular weakness noted in patients who have suffered radiation injury.

The early Los Alamos accidents were reviewed and some follow-up data were given. The man who survived the largest dose in Los Alamos, about 10 or 11 years later developed rather rapidly severe myxedema and also had a myocardial infarction. He has survived both these difficulties and is now getting along well on replacement thyroid therapy. Another patient who had a fairly high dose in Los Alamos continued to go along very well and recently reached retirement age still maintaining very good general health and vigor.

- 2. The zero-energy reactor accident at Vinca B. Pendic
- 3. Treatment of exposed persons in the zero-energy reactor accident H. Jammet
- 4. Follow-up observations on persons exposed in the zero-energy reactor accident at Vinca S. Hajdukovic

Much of these three reports was a repetition of the published information on the Yugoslav accident. Dr. Jammet now emphasizes the relatively high neutron component which recent dosimetry studies have brought out. He believes that the four patients who survived after bone marrow treatment would have died without it. There is a great lack of knowledge in reference to neutron action for different neutron energies. He points out that Yugoslavian patient V had about the same dose as the others, but had a much more profound illness especially in reference to damage to the gastrointestinal epithelium.

specific?

The follow-up on the Yugoslav accident victims was of some interest. The patients continued to have a slight reticulocytosis of from 0.5 to 1.7 percent for several months. Electroencephalographic studies in the surviving patients showed slight abnormalities characterized by low voltage and slight instability. There seemed to be a lack of the usual individuality of patterns expected in a group of five patients, and the tracings all looked remarkably alike. Basal metabolic rates are normal. Eye changes were of some interest. Lens opacities developed and then decreased. They are no longer significantly present. The female patient has had persistent menstrual difficulties with, in general, excessive menstrual bleeding. In the male patients the sperm counts are still very much depressed. The peripheral blood shows a slight lymphopenia. Yugoslavian patient M still has an eosinophilia of over 900/cu mm. The patients complain some of fatigue and

neuro-circulatory instability. There are questionable myocardial changes in two patients; one of these had the changes before the accident.

Dr. Latarjet emphasized the profound damage to the epithelium of the G.I. tract and bladder in the Yugoslavian patient who died. Dr. Killmann wondered whether the number of cells given to the Yugoslav patients could have reproduced fast enough to produce the hematologic responses attributed to the graft. Dr. Woodruff emphasized the use of sex chromosomes as markers, but would prefer to use a tissue culture technique which would allow observation of the chromosomes instead of the clubs on the mature neutrophils. Dr. Koller pointed out that the decrease of donor cells in the Yugoslavs might not be evidence of an immune rejection phenomenon but simply a relative overgrowth of the patient's own marrow precursors. He suggested that skin grafts might help establish the immune status in relation to the previous donors. Dr. Gopal-Ayengar suggested that testicular biopsy would be of interest and that one might look for chromosomal defects in the sperm-forming cells. Dr. Loutit stressed the need for the rad distribution as well as the total rad dose in these cases.

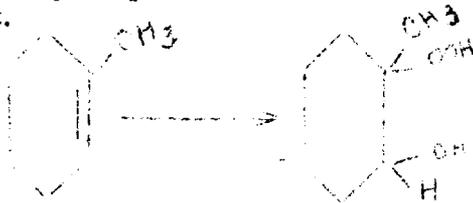
(During the conference several people expressed the opinion that patients do not all respond the same to a given dose of radiation. While this is obviously true, I think that it has been perhaps over-emphasized. The information available seems to me to indicate that the response to radiation is rather more predictable than response to most other injurious agents.)

It was pointed out that stomatitis was a prominent symptom in both the Yugoslavs and the Lockport victims. The Lockport victims had a surprising lack of nausea, even while they were vomiting. They had an increase in adenase in the urine. In the Yugoslav accident the dosimeters were saturated and therefore useless. Improved dosimeters are being made available.

*Interesting!*

Dr. Latarjet seems to feel that antibiotics are all in a sense antimetabolites and that for this reason they may inhibit recovery of the bone marrow and should be used with great caution.

Latarjet reported studies on the nucleic acids of the blood for evidence of radiation effect.



Ekert in Latarjet's laboratory has found a linear relationship between production of this compound and radiation dose.

Cytosine on the other hand yields isobarbituric acid.

Tuesday morning: Studies on acute radiation syndrome in man and its treatment after radiation accidents.

Discussion leader: E. Kowalski

1. A case of radiation fatality resulting from a massive over-exposure to neutrons and gamma rays

T. L. Shipman

This was a very lucid account of the fatal Los Alamos that occurred at the end of 1958. The whole body dose was over 3,000 rads, the front of the chest 12,000 rads. After acute neurological and G.I. symptoms and shock, the patient showed some return of consciousness but died at 35 hours after having been vigorously treated for shock. The

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granulocytes rose to a high level and lymphocytes completely disappeared. Autopsy showed evidence of pronounced cardiac damage; changes in the brain were not prominent.

There was considerable interest in the problem of the rather large amount of fluid the patient had received (about 14 liters) with very little urinary excretion. Dr. Latarjet questioned that direct cardiac damage could have been an important cause of death, because he felt that the life span of the cardiac cells was so long that no significant number of mitoses could have been damaged in such a short interval of time (perhaps I do not have this point entirely clear). Another very interesting point was the presence of a positive Coomb's test in a patient after radiation. Some interest was expressed in the rapidity of the development of rigor mortis. This developed within two hours after the patient's death and was more pronounced in the more radiated parts of the body. It was said that the creatine in the urine of this patient was normal by one method, but more than 50 times normal in Hempelmann's laboratory. No hemoglobinuria was present.

?

Latarjet, in considering the recent fatal Los Alamos case, thought that instead of direct cardiac effect we might be looking at a biochemical effect. He points out that the total energy delivered to the patient would have raised the body temperature only a fraction of a degree.

2. The basis underlying clinical diagnosis of acute radiation sickness. G. A. Zedgenidze

This dealt largely with the ordinary radiation sickness seen after clinical external radiation therapy. The location of radiation ports influenced the incidence of nausea and vomiting; abdominal ports were most likely to produce this effect, thoracic ports somewhat less likely, and head and extremity ports still less likely. No great effort was made to stress central nervous system effects, but "functional" changes were considered important. Some emphasis was put on these changes in function, especially of the G.I. tract where gastric retention, etc., result from radiation effects. Much of the experimental work was done with rabbits.

→ Loutit said that changes in gastric function had been demonstrated with doses as low as 25 r. There was some discussion of protective drugs and their relative effect on normal tissues and tumors, but Dr. Zedgenidze had no data on this.

WBC  
man

There was some consideration of the later stage, when marrow depression is important. Dr. Zedgenidze reported that concentrated leukocytes were used with great success in Moscow for therapy of this condition and this claim was strongly supported by Dr. Kowalski, who said he had observed the benefit. In France, Maupin has been unable to achieve therapeutic results with concentrated white cells, it was reported. Woodruff referred to work indicating that isologous thoracic duct lymphocytes will facilitate survival of lethally irradiated rats.

Tuesday afternoon: Studies on acute radiation syndrome in man and its treatment after radiation accidents

Discussion leader: A. R. Gopal-Ayengar

1. Dosimetric considerations in criticality exposures J. A. Auxier

This was a very interesting report. The experiments made by the group who went to Yugoslavia to reevaluate the dosages in the Yugoslavian exposed group were reported.

They felt that they were able to reproduce the exposure conditions quite satisfactorily, and to establish doses within a reasonable range of accuracy. The following doses were given for the Yugoslav group:

Individual Doses (All Values are in Rad Units)

Individual	Charged Particle Dose	H(n, $\gamma$ )D Gamma Dose	External Gamma Dose	Total
H	66	99	158	323
V	89	133	214	436
G	90	135	189	414
M	87	130	209	426
D	91	136	192	419
B	45	67	95	207

Some questions were raised about the use of the radiosodium content of the blood and how corrections were made for biologic turnover of sodium. It was stated that these were based upon similar studies in the burro. It was said that the discrepancy with the earlier dosage estimates, which were quite different, had to do with evaluation of the softness of the fast neutron spectra.

2. Acute radiation effects on man revealed by unexpected exposures S. A. Killmann

*Mitotic index*

This was a report of work done at Brookhaven, much of it relating to the Y-12 accident group in Oak Ridge. The first main item was the mitotic index. It was pointed out that this measurement depends upon (1) mitotic time, (2) the ratio of red cell to white cell precursors in the marrow, since there are relatively more mitoses in the red cell precursors, and (3) the relative number of cells at a stage of maturity enabling them to undergo mitosis. In spite of these problems, the crude mitotic index is of value as a measure of radiation injury.

*#2 + #3*

The second technique was the study of DNA synthesizing cells by tritium-labeled thymidine. The third was the measurement of BAIRA in the urine.

Killmann also presented the technique of treating dogs using platelet transfusions and antibiotics which makes it possible to carry them through doses of radiation that would be otherwise lethal. A chart was shown which indicated that at a certain dose range of radiation the mortality could be reduced by supportive measures including antibiotics and platelets; that there was a higher dose range of radiation exposure in which there was no form of effective therapy, and that with still higher doses bone marrow grafts could be used. This chart, however, was based upon theoretical considerations and the main experience of the authors was in the effective treatment in the dose ranges where antibiotics and platelets would reduce the mortality.

*Important*

In discussion this last chart was somewhat attacked. Dr. Mathe' said that he felt a graft was possible with a sub-lethal dose of radiation, and Dr. van Bekkum said that he felt there was little evidence for the existence of this therapeutic gap in man. Mathe' pointed out that in recent work Uphoff found it very difficult to show increased mortality as a result of marrow administration at sub-lethal radiation levels unless very carefully selected combinations of mouse strains were used. Dr. Kay mentioned a drug called Celbenin which is a new penicillin. It seems that organisms cannot become resistant to it and that it is not destroyed by penicillinase. Its use as an aerosol gets rid of staphlococci. (This is reported in LANCET, Vol. II, page 564, Sept. 10, 1960, and is presumably the same as the "Staphcillin" being distributed in the United States.)

Dr. Tocantins said that he had some doubt about the value of asepsis in treating patients with marrow aplasia since many such patients died of infections with organisms already present in the body, such as E. coli, staphlococci, and pseudomonas. He was somewhat less opposed to the prophylactic use of antibiotics than were Dr. Mathe' and Dr. Killmann. Dr. Tubiana, however, said that he would like to know the nature of the infecting organism before giving an antibiotic to such patients. There was some discussion about whether an ordinary man was more comparable to the healthy dog achieved in certain very modern and well cared for animal laboratories, or more comparable to an ordinary dog with no special care.

Wednesday morning: Therapeutic whole body radiation and tissue transplantation in man

Discussion leader: J. W. Ferrebee

1. Secondary syndrome: a stumbling block in the treatment of leukemia by irradiation and transfusion of hematopoietic cells

*G. Mathe' why graft + rad helps.*

*~ 20000 rads to destroy*

Studies with mouse leukemia have shown that to destroy all leukemic cells it would require much higher doses than the mouse could tolerate, even with a successful bone marrow graft. To the direct antileukemic effect of radiation may be added an antileukemic effect based upon immunologic mechanisms when the marrow graft is from a mouse of different strain. This effect, however, appears to be demonstrable only when the number of leukemic cells is low. This is a justification, from animal experiments, for attempting to treat human patients during periods of remission. The secondary syndrome, however, which is a reaction of the graft against the host, interferes with successful therapy on this basis. In the mouse experiment, efforts to separate the antileukemic immunity reaction from the secondary disease have been attempted by using hematopoietic cells from fetal donors, by using a mixture of marrow and lymphoid cells, and by using cells from donors immunized by a leukemic graft. Efforts have also been made to treat the secondary syndrome with amethopterin, which is also of course antileukemic. These studies had been, in general, disappointing from the point of view of therapeutic effect.

*820 rads WBR*

Six children with acute leukemia in remission have been treated with doses from 820 to 950 rads followed by transfusion of homologous bone marrow. Two died in a state of aplasia without having evidence of successful marrow graft. In four others, myeloid repopulation was seen due partly or wholly to proliferation of the injected cells. This was followed by development of a secondary syndrome which was early and fatal in two of the patients and somewhat later and transient in two others. The patients who died as a result of the secondary syndrome had received the donated marrow from the mother. The syndrome was characterized by severe diarrhea, vomiting, and erythrodermia. One of

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*2 aplastic  
2 regner -> Secondary disease  
2 regner -> transient + sic + Dis*

them had a candida infection and virus pneumonia. The marrow was restored but the lymphocytes were practically absent. The other had severe liver necrosis which may have been related to a previous viral hepatitis. The patients who died with secondary syndrome had no evidence of persistent leukemia.

Dr. Woodruff commented on experiences in treating patients with ovarian cancer by use of large doses of alkalating agents and then autologous or homologous marrow. He presented a slide on one patient who received thiotepe, 30 mg/day, for a total of 7 days out of 11. This patient then developed severe marrow depression and was given marrow from the brother. Although there was no proof of a chimera, Dr. Woodruff felt that the marrow donation helped recovery. Dr. Kurnick commented upon the length of time it requires for hematologic improvement to occur after the administration of the marrow and agreed with Dr. Woodruff's interpretation. No one seemed to wish to argue the question of whether the patient might have recovered without any marrow administration. Some comments were made about virus infections and it was emphasized that many people believe that they are important in relation to the secondary syndrome, but no one has much information on it.

*Ideal Donor?*

Choice of the ideal marrow donor was discussed. Should it be a member of the family? a patient matched according to red cell types or leukocyte antigens? or perhaps a mixture of marrows from several sources? No one had a good answer to this. Dr. Killmann emphasized the importance of leukocyte antigens.

Dr. van Bekkum said that in mouse given rat bone marrow it was possible to partially prevent the secondary syndrome by having aureomycin in the diet of the recipient for a long period of time. It was also somewhat helpful to use a diet high in cereal. He could not demonstrate this effect in the monkey, however.

Dr. Congdon discussed the pathology of secondary disease and said that it was compatible with the general picture of auto-immune phenomena and not greatly different from that of lupus erythematosus.

*Many mice dying or leukemizing post*

Dr. Latarjet reported on some work done by Dr. Duplan. He studied AK mice which had a spontaneous incidence of leukemia of about 80 percent. When they were exposed at an age of three months to 750 r and then given embryonic liver from the same strain, there was still the same incidence of leukemia but it developed about three or three and one-half months later than in the untreated animals. When the mice were radiated with the same dose and given marrow from mouse strain I7, the leukemia occurred at the same time interval as in the normal mice, but there was only one-half the usual incidence. Some of the leukemias were AK type and some I7 type. This seemed to indicate that the leukemia was caused by an agent that could shift to the donor type cells. (In this particular set up the secondary syndrome was not prominent.) Dr. Latarjet felt that the best time for giving homologous marrow in the human was probably ten days after radiation. Dr. van Bekkum felt that some degree of delay made rejection less pronounced. He pointed out that the secondary disease had been well described in the mouse, rabbit, monkey, and dog and that in all these the changes were quite similar with prominent gastrointestinal effects, lymphatic changes, and evidences of increased susceptibility to infection.

Dr. Tocantins said that aspirated marrow might be useful for autologous purposes but was not suitable for therapy when a homologous donor was used. He feels that a 0.1 percent sequestrane solution buffered with phosphate was the ideal agent for suspending the marrow. He said that studies had shown that in mice frozen autologous marrow from previously prepared donors had failed to transfer the protective effect against tetanus toxin, but when the same experiment was done without freezing the marrow this protective effect

*Dr. Kurnick  
Circumferential  
Sodium  
Proc  
for freezing*

was transferred. Dr. Kurnick said that in trying to prepare a suspension of marrow for use, added protein would improve the apparent viability of the cells. Dr. van Bekkum wondered why Dr. Tocantins did not favor the use of heparin, and Dr. Tocantins said that it was because there was more clumping with heparin. Dr. Thomas thinks that in seques-trene cells lose ability to synthesize DNA and he believes that heparin may be the best anti-coagulant but feels that the preservatives present in it may be a disadvantage. He has obtained special heparin free of preservatives. Dr. Tocantins thinks heparin is satisfac-tory for aspirated marrow but not for surgically obtained marrow. Dr. Woodruff said that the clumping effect and the debris seen in preparations where heparin was used were due to the presence of calcium and magnesium. These lead to the production of so-called nucleoprotein slime. Dr. Kurnick avoids the use of electrolytes entirely and just uses a glucose solution along with heparin. He uses an amount of heparin that leaves him eventually with 2 units per milliliter of aspirate. Latarjet mentioned other media, Philpot's, for use in marrow that is to be frozen. Dr. Congdon mentioned that they had made efforts with Sorbitol, Ribitol, Choline, and amino acids, all of which had some protective effect when the cells were frozen. He thinks that perhaps it is best to use a mixture of these, and recommends slow freezing to -25 degrees, then fast freezing to the storage temperature. Thawing should be done rapidly.

Dr. Woodruff discussed the laws on availability of tissues. He feels that it is very important for those interested in this problem to sponsor good laws which will not be res- trictive in their action. Some people felt that perhaps no laws at all would be better, since some degree of limitation is likely to be included in almost any law.

2. Problems in procurement, preservation and administration of human bone marrow

L. M. Tocantins

Dr. Tocantins reviewed the extensive work he and his group have done on obtaining fetal and autopsy marrow. He described in detail the techniques they have worked out for freeing the marrow from the bony framework.

He believes that aspirated marrow is of very limited value because of the small number of cells obtainable and the relative lack of primitive marrow elements.

3. Whole body irradiation for renal homotransplantation

M. Tubiana

This was a description of four separate attempts in kidney grafting. 1) A kidney was available from a non-identical twin brother. The patient was given 250 rads and then 8 days later another dose of the same magnitude. The graft is doing well at 18 months. The kid-ney functions well. The patient's blood pressure and fundi have improved greatly. At 6 months after the graft an operation had been done to remove the two diseased kidneys which had both been left in. This seemed to help the patient's general condition. 2) A man with bilateral cancer of the kidneys. This patient had had bilateral nephrectomy before the graft was undertaken. He was given a kidney from his sister, after 400 rads plus 200 extra rads to the splenic region. The transplanted kidney did well but the patient died 5 months later from hepatic metastases. 3) A woman age 22 with nephritis was given a kidney from her sister. She was given 200 rads and then 3 days later 230 rads. The kidney did well for 3 months then she had evidence of an immunologic reaction and was given 100 r of extra total body irradiation. The renal function improved and she is doing well. 4) A woman 25 years of age was given a kidney from an unrelated donor of similar blood type. She had been given 400 rads with 200 rads extra to the spleen. She got along well for two months and then the kidney function decreased. She was given a similar number of rads, plus some extra radiation to the kidney. The renal function is rapidly improving and she is now doing well.

*1) take  
2) take  
3) take  
4) take*

*Tubiana  
reaction can be  
depressed by  
more X-rays*

Dr. Tubiana believes that it is desirable to have a member of the family as a donor. It is clear that the secondary rejection phenomena can be helped or controlled by further radiation. In the first case presented, where the twins were non-identical, there may have been exchange of compatibilities in utero, although skin grafts between the two were rejected. The patient with the renal cancer had been carried on the artificial kidney for several days. In one case a decapsulation operation was necessary 5 days after the transplant. They now do a routine decapsulation procedure with each transplant. If scars have not healed by the time of greatest leukopenia, there will be no healing until marrow regeneration occurs.

Great emphasis was placed on a germ-free room which has been developed to a rather high level. This involves careful use of gowns and sterile clothing, preparation of the hands, etc. The room has a positive pressure so that all of the organisms that get in tend to be blown out. Ultra violet is used extensively to sterilize air. Special filters and air conditioning are used. Special food preparation techniques are used. He felt that their experience showed that a patient could be maintained even under very bad circumstances of surgery, etc., if this sterile environment was available. One of their patients had a very long complicated course but eventually recovered. These patients develop fever during periods of severe marrow depression, even when they are in the controlled environment and have no apparent infection.

The attendants for the controlled environment facility hold their hands in alcohol for 3 minutes for sterilization. The patients and attendants wear dark glasses as protection against the ultra violet light. Television helps to keep the patients amused, and the television machine is kept sterile. A glass wall is used so that the family can be near the patient on the other side of the wall, and they can communicate with a microphone set-up. A bacteriology is done on the patient before treatment and antibiotics may be used for specific infections. In other cases, administration of bacteria is used to balance the flora. They do not wish to change the bacterial pattern too much and very much prefer not to use antibiotics during the period of greatest marrow depression unless they have a specific infection identified. Treatment of these infections may have little effect on the fever.

*Severe*

One of their patients had splenomegaly after the radiation and again had a splenic enlargement with a second course of radiation. EEG studies have been done and some changes seen. They had seen examples of icterus after irradiation and are very much interested in the mechanism of it. They had not seen total epilation in any of their cases. Dr. Tubiana showed hematology charts which were very hard to follow. It appeared that the leukopenia was more severe in these patients than in the Y-12 patients. The total white count went below 500 in most of them. It appeared that the granulocytes fell quicker than in the Y-12 cases, and what is most interesting, in at least one or two cases seemed to recover somewhat earlier. This is somewhat complicated, though, by the fact that two doses of radiation were sometimes used. Incidentally, he said that he felt that the effect of two doses spread apart by a few days was about the same as the whole amount given on one day. Dr. Tubiana believes that the heterogeneity of the dose is important in the accident cases.

In one instance a second dose of 100 r given several weeks after the first dose had about as much effect on the hematologic picture as a 300 or 400 r dose had originally; although there was no secondary epilation. Perhaps the total number of cell precursors was small, and there were relatively more cells in the process of division. This result conflicts with certain animal data. In another case evaluation of a second dose was complicated by recent therapy with 6-mercaptopurine.

Dr. Killmann suggested that the rapid fall in WBC after radiation might be related to the uremic state of the patients.

There was no meeting on Wednesday afternoon but the morning meetings had run quite long.

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Thursday morning: Therapeutic whole body radiation and tissue transplantation in man

Discussion leader: M. Woodruff

1. Procurement and preservation of human foetal tissues and their use in the treatment of injury to blood-forming tissues

H. E. M. Kay

Dr. Kay pointed out that fetal grafts might <sup>retain</sup> regain tolerance to hosts and thus might produce less danger of secondary disease than adult marrow grafts. Animal experiments, however, indicate that immune reactions are by no means eliminated by fetal graft. Fetal grafting probably leads to more restoration of lymphoid tissue than does adult marrow. Dr. Kay mentioned that the social customs and laws vary in different countries in relation to the possibility of obtaining and utilizing fetuses. Their program at the Royal Marsden Hospital has been able to obtain 630 fetuses of from 12 to 28 weeks of gestation. The tissues from these fetuses, particularly the kidneys and lungs, are valuable to people interested in tissue culture work. They use the Schrek eosin test to demonstrate the viability of the liver cell suspension. They have also studied the intracellular granular oscillations by means of a phase microscope. A new test developed at their place involves the use of acridine orange and a fluorescence phenomenon which looks quite promising as an index of viability of cells. They do tests for bacterial and viral infections in the fetuses. They use meshes to separate the cells and have considered using tryptic digestion as recommended by tissue culture workers.

*Acridine orange*

Dr. Kay showed pictures of their device for freezing the tissues. There is an outer vessel containing alcohol and dry ice. An upper container also containing alcohol is inserted into the lower one at different levels so that greater or lesser portions of the upper are in contact with the alcohol of the lower vessel. A stirrer is present in the upper container and a thermocouple for measuring temperatures. The ampules of tissue are inserted in the upper container and temperature is controlled by the degree of lowering into the lower. Preservative materials they are using include 15 percent glycerol. They get 30 million to 15 billion cells from each fetus liver. This includes a lot of hematopoietic tissue. At 13 weeks the liver is about two-thirds hematopoietic tissue, and at 26 weeks about one-third hematopoietic. They estimate that this tissue might be one-half as effective as mature marrow and that they lose about two-thirds by freezing; thus 30 average fetuses would produce 20 billion cells for one treatment and this is the amount they think might be effective. Older fetuses are harder to get, may have lost their tolerance. Immune responses develop about two or three weeks after birth, regardless of the state of prematurity at birth, but in some respects, for example rejection of skin grafts, the immunity is present earlier. Fetal graft tissues will not necessarily remain tolerant to host antigens unless "immune barriers are low." Minor secondary disease may be seen even in a successful case. This might relate to the number of immune cells. Dr. Kay presented the following concepts as a highly tentative suggestion:

$$\frac{Md \times Ld \times X}{Lr} = \frac{1}{Ad - Ar}$$

This indicates possibility of preventing early radiation death with fetal tissue.

$$\frac{1}{Ld} \times \frac{X}{Ar - Ad} = 1$$

Chances of avoiding secondary disease.

- Md = donor marrow cells
- Ld = lymphoid cells, donor
- Lr = recipient lymphoid cells remaining
- Ad = antigens of donor
- Ar = antigens of recipient

*note*

He said that pooled adult grafts have graft-versus-graft reactions. This may not be true of fetal or newborn tissues. Failure of the human graft may be due 1) to resistance of the R.E. system, or 2) high immune barriers. A fetal marrow donation may liberate ATF and produce pulmonary embolism. He presented an example of a patient with seminoma who had had much radiation previously. He was given 500 r of total body irradiation and 20 billion fetal cells. There was no evidence of success. Fetal cells were divided into several doses. Another case, a patient with lymphosarcoma with much previous treatment, was given fetal liver cells without any recent radiation. There was a reticulocyte response and a platelet response. Some fetal type red cells were seen as demonstrated by anti-N serum. The use of fetal liver avoids introducing mature red cells that contaminate the tests for donor type cells. Dr. Kay also mentioned the case reported by Bridges in which thiotepa 250 mg was given over a period of 9 days. Fetal cells were then given. When the blood was studied the top layer, rich with reticulocytes, was positive for donor type cells. Dr. Kay believes that in no case has a sufficient number of fetal cells been given after an adequate dose of radiation. He uses the word billion in the American sense meaning  $10^9$ . (Apparently the British sometimes mean  $10^{12}$ .)

Dr. Thomas commented that they had seen deaths in dogs after fetal tissue administration and that these could be reduced by very careful screening and washing of the cells. In 5 patients and 12 dogs they had tried to use fetal tissue, but had no evidence of successful grafting. They used screens as fine as 40 microns. In some cases they have seen death from shock and some from pulmonary emboli when they used fetal tissue. Dr. Tocantins said that it was possible to separate liver cells from hematopoietic cells by their sedimentation behavior, but that the important stem cells might stick with the liver cells.

2. Bone marrow transplantation in man

E. D. Thomas

Dr. Thomas reported the cases that had previously been published. He now feels that the only successful grafts were those obtained from identical twin donors and a single early case in which a patient with lymphocytic leukemia was able to accept foreign marrow, more because of a lack of normal immune response associated with the disease than because of the radiation given. (This case, in which the foreign cells are C-positive in a C-negative environment, must be interpreted as showing a most remarkable rapid red cell production if the cell quantitation data are correct.)

He has seen one human case in which the injected cells seemed to contribute to pulmonary embolism and cor pulmonale.

The identical twin studies are of great interest. Radiation doses *cf cases* used were 850, 1140, 1600, and 2000 r. The marrow given from identical twin showed beginning dose evidenced by hematologic response at a period of from 8 to 12 days after administration. All of their patients except one, I believe it was the one with the 2000 r dose, lived through the immediate postirradiation period and had remissions in leukemia but remissions were not very long lasting.

Dr. Thomas' talk brought forth rather pointed questions by the French workers who seemed to feel that it was impossible that patients had survived such large doses of radiation. They asked about the degree of epithelial reaction (which was not apparently very profound) and raised questions about whether or not the radiation dose was uniform. They felt that a penumbra effect might have influenced the results seen, but Dr. Thomas reported that the field was large enough to encompass the whole body. In contrast to the French group, Dr. Loutit said that he thought that the results were quite believable. Considerable discussion centered on the dose rate of the radiation. Thomas' cases were treated at rates of 0.5 to 2.5 r/minute.

Dr. Thomas reported that in one patient the serum amylase went up very strikingly after radiation during the first 24 hours.

He also pointed out that the administration of blood along with bone marrow may cause trouble because of immunologically competent cells in the peripheral blood. *RBC given!*

Dr. Tocantins remarked that in patients with E. coli septicemia the blood may be hypercoagulable and that this might explain the appearance of pulmonary emboli from marrow in a patient with this complication. He thinks that marrow intravenously is usually well tolerated, but that it would cause great trouble if given intra-arterially. The lungs apparently offer a protective mechanism for filtering out large particles without excessive damage. Dr. Vetter pointed out that the time in Dr. Thomas' cases between injection of cells and evidence of appearance of donor cells in peripheral blood was longer, (3-12 days), than in the Yugoslavs (5 - 6 days).

Thursday afternoon: Bone marrow transplantation in man and animals

Discussion leader:

P. C. Koller

1. Autologous bone marrow transplantation in patients

*Partial body irradiation*  
N. B. Kurnick

Dr. Kurnick reported mainly on a group of patients given external radiation to large ports but not to the whole body, who were also given autologous marrow which had been drawn before the beginning of the radiation treatment. He felt that the autologous marrow had 3 effects, 1) it hastened the hematologic recovery, 2) it caused hyperplasia of the bone marrow, and 3) it caused repopulation including areas of marrow known to have been hypoplastic for long periods because of local external radiation. In addition to these patients treated by autografts he had one patient who was given marrow from an identical twin and who tolerated a large amount of P-32 (40 millicuries in 10 days).

In general, the white blood counts rose by more than 2000 from the initial values in from 10 to 28 days after the administration of the autografts. One patient who had chronic leukopenia from previous radiation for seminoma was given marrow from a twin who may or may not have been identical. This was followed by a prompt rise in the blood counts and also a development of eosinophilia which had been very prominent in the donor but not previously present in the recipient. Dr. Kurnick had no success in patients with aplastic anemia and in a patient with acute leukemia he was unable to destroy the leukemic cells even with 890 r of radiation. Two of his patients had cerebral vascular accidents during the infusion of the bone marrow but the mechanism of this was not understood.

In his first group of patients, with the chronic pancytopenia from radiation, he found that if marrow was given later, after a fairly good peripheral blood recovery had been established, it was ineffective in correcting the general hypocellular marrow state. It seems necessary to give it shortly after irradiation to produce this repopulation effect. He feels that this difference in effect is not related to problems of storage of the cells, or duration of hypoplasia.

He has no true controls but he has some patients who had somewhat less severe depression from their radiation therapy, and in these the hematologic response was less rapid than in those who received the autografts. He thinks that in man the precursor cell is fixed and does not travel unless given intravenously, while in mice there is a strong tendency for spontaneous repopulation from areas of undamaged marrow. He handled his

aspirated marrow with a 50 percent diluent consisting of glycerol and Osgood's mixture. Just before giving it he adds 1/3 volume of 35 percent glucose. This makes the final preparation 7 times isomolar with 10 percent glucose and 10 percent glycerol at the time of infusion.

Dr. Tubiana had some data on bone marrow effects of local radiation. He also agreed that generally the marrow stays hypoplastic for a long time at the site of local irradiation. He has studied this some with Fe-59. They have used the new aseptic environment technique to make possible rather high doses of conventional radiation therapy.

2. Fate of skin grafts in irradiated rabbits treated  
with homologous adult or fetal hematopoietic tissue

K. A. Porter

This discussion included an evaluation of the rabbit as an experimental animal for this type of study. An important disadvantage is that the rabbit sometimes dies very quickly from a shock-like state after radiation of the level needed for the experiment. There are also rather profound gastrointestinal manifestations after irradiation. By dividing the dose into three daily doses (600, 500, 500 r) it is possible to achieve a satisfactory marrow graft experiment using sex characteristics as markers. Antibiotics were required. The use of liver cells from 20-day rabbit fetuses and bone marrow from adult rabbits was undertaken. It was possible to produce chimeras from both groups with adult marrow,  $1 \times 10^9$  cells, fetal liver  $1.4 \times 10^9$  (pooled from 4 female fetuses). The homologous fetal liver tissue was as good as adult homologous marrow in preventing early death and produced much less evidence of secondary disease. There was some later reversion to donor type cells in those treated with fetal cells. Skin grafts would persist on both types of chimera but would last longer on those animals that had received the fetal liver cells. The main difference in the acceptance of skin graft between the liver-treated and the marrow-treated animals was seen during the first 14 days after irradiation. At about 5 weeks both groups regained ability to reject grafts. Dr. Porter referred to a technique for sex identification of lymphocytes (Riis, P. Acta Haematol. 18, 168, 1957).

van Bekkum said that it was agreed that in general fetal tissue is usually inferior (more cells required) to adult marrow for an initial take, but better in reducing secondary disease.

3. Marrow graft experiments in the dog

J. Ferrebee

This was a discussion of the efforts that had been made by the Cooperstown group to graft marrow in the dog. They have been able to achieve survival after very large doses and apparent successful chimeras in a few dogs out of quite a large number studied. Many of the dogs died of what seemed to be secondary disease but had prominent evidences of infection.

Dr. Ferrebee's group has become increasingly interested in the possibility that the presence of peripheral blood taken from the marrow donor or from other donors may handicap the success of a graft experiment and have used irradiation of the peripheral blood while in the transfusion bag at a dose of about 1000 r. This irradiation does not appear to impair the usefulness of the blood for therapy of the pancytopenia of radiation injury.

Friday morning: Studies on acute radiation syndrome in primates and experimental therapy.

Discussion leader:

E. Paterson

1. Pharmacological characteristics and efficiency of certain mercaptoamines in preventive treatment of radiation disease

A. M. Rusanov

This talk was given in Russian and seemed to be quite well presented. It described many pharmacologic investigations on radiation protective drugs, especially AET.

2. Treatment of irradiated monkeys with autologous and homologous bone marrow

D. W. van Bekkum

These studies included both autologous and homologous experiments. At doses of from 850 to 925 r, autologous marrow seemed highly effective in allowing survival of the animals. The marrow was removed from the femur by a trocar inserted into the knee joint and was re-infused about 2 hours following the radiation. In the homologous experiments, at doses of 550 to 935 r, it appeared possible to approximately double the survival of the animals with homologous marrow, but they quickly developed what appeared to be secondary disease and died at around 26 days after the exposure. (The control animals died at about 15 days after exposure.) The marrow was given 24 hours after exposure and the sex marker was used in an effort to show the proliferation of donor cells and there was evidence that such proliferation did occur. The cells used for grafts were suspended in tyrode's solution, and strained through a gauze without additional handling. Most of the animals were given antibiotics including penicillin, streptomycin, terramycin and chloramphenicol during the period of radiation depression of the marrow. Hemorrhagic manifestations were prominent as a cause of death in the fatally irradiated animals. The LD<sub>50</sub> is believed to be about 600 to 650 r. In a group of animals radiated at doses of 650 to 1050 r, only the higher dose levels gave any G. I. symptoms. Those that developed what appeared to be secondary disease had dermatitis of the face and pronounced diarrhea without any infecting organism to account for it. For the autologous experiments, cell numbers in the range of 2.2 to 12.9 X 10<sup>6</sup> were used.

3. Experimental treatment of radiation injury in monkeys

D. R. Anderson

It was found that by combining cysteine and AET it was possible to effectively reduce the mortality from radiation. They were able to achieve homologous graft using immunologically typed erythrocytes as a marker, and as much as 40 percent donor type cells were present on the 28th day; however, there were no long term survivors and secondary disease was again the prominent feature. The animals that had received homologous marrow had high levels of SGP transaminase and also of alkaline phosphatase.

In commenting on this paper Congdon brought out the evidence that blood impairs the usefulness of marrow and causes an accelerated secondary disease. 950 r of radiation to blood will eliminate the detrimental effect. The peripheral blood presumably has antibody forming cells present. If isologous blood is mixed in vitro with the marrow it destroys the therapeutic value of the marrow.

Dr. Killmann brought out that heavily irradiated animals are the best source of erythropoietin and he therefore believes that erythropoietin would have no value in the treatment of radiation injury. He emphasized his interest in the use of absolute reticulocyte values rather than percentage levels. Congdon reported that there is increased tritium-labeled thymidine in the germinal centers of lymph nodes after antigenic stimulation. Thomas said that the dog has only two viruses while the monkey has multiple viruses and he wondered about the importance of viral disease in the monkeys that died apparently of secondary disease. van Bekkum said that inclusion bodies were present in some animals of this group.

Friday afternoon: Problems for future study

Discussion leader: C. C. Congdon

1. Effects of whole body irradiation in man and other mammals and their modification by experimental and therapeutic means

J. F. Loutit

This was a carefully thought out and balanced presentation of the present status of the whole problem of radiation accidents and their management. It was pointed out that the protective agents which must be given in advance are likely to be of very limited practical usefulness. After an accident has occurred, the initial problem is of course to establish the dose. For very low doses, in the subclinical level, the health physicist may be in the best position to do this, but with higher doses, the clinician is then most qualified to determine the extent of the injury. Dr. Loutit says that he doubts that the human is so variable in response to radiation as compared with other mammals, as is often stated. He believes that the apparent radiation is due to the different types of radiation and the problems of dosimetry in the various accidents that have occurred. He believes that the dose should be stated in the rad unit, but that the distribution, degree of homogeneity, and duration of exposure are also important. He spoke of the various biological evidences of radiation injury such as fall in lymphocytes, changes in amino acid and creatine excretion, and the time required for development of various clinical manifestations. He seemed rather pessimistic about the specificity and reliability of the various laboratory tests for indicating the severity of radiation exposure. It is his estimate that about 1000 rads would be required to produce completely irrecoverable destruction of the hematopoietic and immunogenetic tissues. In such a circumstance, if a graft could be achieved it would probably be permanent. At lower doses, any graft achieved is likely to be temporary. He pointed out that the harmful effect of homologous marrow seen in some animal experiments at certain sublethal doses is not a universal finding and perhaps has been exaggerated.

Following this there was a rather general discussion on radiation injury and therapy.

Summary of the Important Points in the Conference

1) It appears that patients can tolerate larger doses of radiation than was previously believed. This is possible because of improvements of therapy, including supportive treatment, use of antibiotics, prevention of infection, and use of bone marrow. The fact that these survivals have been seen after very high doses alters somewhat the prognostic implications of certain clinical and laboratory tests. This does not mean that the clinical and laboratory tests were misleading in indicating the dose received, but simply that the lethal range has been pushed up by therapy. 2) The development of effective means of preventing exogenous infection appears to be a significant advance in the therapy of radiation injury, which may be applicable to various accidental and therapeutic situations. 3) Successful homologous kidney transplants have been achieved in patients with some uniformity with the use of radiation to depress the immune mechanism but without any attempt to transplant marrow. There is continued emphasis on the immunologic aspects of these problems. 4) Secondary disease continues to be an area of major interest. In the monkey given homologous marrow, secondary disease appears very rapidly and is a very serious problem. The importance of possible viral infections and of liver involvement in the secondary syndrome were emphasized.

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