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FIGURE 1. Leukocyte and platelet levels in a patient treated with 0.2 mg. $\text{HN}_2/\text{kg.} \times 4$. Autologous bone marrow was reinfused at time of marked leukopenia and thrombopenia.

FIGURE 2. Leukocyte and platelet levels in patient treated with 0.2 mg. $\text{HN}_2/\text{kg.} \times 4$. Bone marrow was not reinfused in this patient. Platelets rose to $8.0 \times 10^5/\text{cu.mm.}$ in recovery phase.

FIGURE 3. Leukocyte and platelet levels in patient treated with 0.3 mg. $\text{HN}_2/\text{kg.} \times 4$. Bone marrow was not reinfused. Note striking rebound of leukocytes and platelets in recovery phase.

FIGURE 4. Composite data on leukocyte and platelet levels of patients receiving 0.2 and 0.3 mg. $\text{HN}_2/\text{kg.} \times 4$, without bone marrow reinfusion. Note patient HT who received maximal dose and showed one of the earliest recoveries in cellular components.

FIGURE 5. Comparison of decline and recovery of leukocytes in patients receiving 0.2 and 0.3 mg. $\text{HN}_2/\text{kg.} \times 4$. Patients receiving autologous bone marrow are shown at top of the table.

FIGURE 6. Comparison of decline and recovery of platelets in patients receiving 0.2 and 0.3 mg. $\text{HN}_2/\text{kg.} \times 4$. Patients receiving autologous bone marrow are shown at top of the table.

AUTOLOGOUS BONE MARROW TRANSFUSION FOLLOWING CHEMOTHERAPY*

* Paper prepared with technical assistance of Inez F. Miller and Irving M. Jones.

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An aggressive attack in the management of malignant disease by chemotherapeutic means has been hampered by toxic effects of these agents on bone marrow, gastrointestinal tract, and skin. Following reports that shielding of spleen or limbs, or injection of spleen or bone marrow suspensions, protected animals from otherwise lethal doses of irradiation, attempts were made to apply methods to patients.^{1, 2, 3, 4} An extension of this thinking was the use of autologous bone marrow transfusions following chemical treatment for malignancy.

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Previous workers have had experiences with a variety of agents, viz., nitrogen mustard, triethylenemelamine, thiotepea, methotrexate, and cytoxan. In all of the above series no effort was made to label or identify transfused marrow cells and control studies were absent or inadequate. Since the present report is concerned solely with the use of large doses of nitrogen mustard, past experiences with this drug in conjunction with autologous bone marrow transfusion will be briefly reviewed.

In 1959, McFarland, Granville, and Dameshek treated five patients with malignant lymphoma with what they termed "super-doses" of HN_2 , ranging from 0.8 to 1.4 mg./kg. of body weight, divided into three injections.⁵ Autologous bone marrow containing about 2.0 billion cells was reinfused four hours after the last injection. Two patients died during the pancytopenic phase, one on the 11th and the other on the 19th day after drug administration. Changes in leukocytes and platelets in one case were as follows: This patient received 1.1 mg. HN_2 /kg. body weight, following which 2.5 billion nucleated cells were infused. Leukocytes began to fall on the fifth day and were depressed until the 14th day, when they began to rise. Platelet fall was on the eighth day, with beginning increase on the 17th to 18th day. Since the authors showed no control cases of patients treated with similar doses without bone marrow transfusions they were cautious in not attributing hematologic improvement to bone marrow transfusions.

In the same year, Black, Speer, and Stone treated 10 patients with advanced malignant disease with HN_2 in doses ranging from 0.4 to 0.65 mg./kg. in single injections.⁶ This was followed in 10 minutes by re-administration of 18 to 20 ml. of bone marrow which were aspirated from the sternum immediately before HN_2 injection. In four cases marrow suspension was infused by vein, and in the remaining six patients it was introduced directly into the sternal cavity. Leukocytes fell as low as 1,400/cu.mm. in three to seven days, leveled off in 12 to 16 days, and were increased above pre-treatment levels after 30 days. Platelets never fell below 100,000/cu.mm. except in one patient who died. The effects of HN_2 on total number of leukocytes in this study were compared with those of Bierman who administered 0.6 mg./kg. in a single dose.⁷ Black and his coworkers suggested that there was an earlier recovery from leukopenia which proceeded much more rapidly than in untreated subjects. In one case the authors treated a patient on two different occasions with 0.6 mg. HN_2 /kg. body weight, infusing 20 ml. of marrow suspension after each course of therapy. The authors suggested that injection of these small quantities of marrow minimized toxic effects of HN_2 .

In 1960 Kretschmar reported on eight patients with a variety of malignant diseases treated with HN_2 in doses of 1.0 mg./kg. body weight.⁸ In four cases 144 ml. of marrow were aspirated immediately before nitrogen mustard therapy and reinfused intravenously six hours later. In both groups leukocytes began to fall one to three days after HN_2 , reached minimal levels on the seventh to ninth day, and remained below 1,000/cu.mm. through the 14th day, after which they began to rise. White cells rose to 4,000 to 5,000/cu.mm. on the twenty-eighth day. There were no differences between control and bone marrow treated patients in decrease, minimal levels reached, or time and rate of recovery of white blood cells. Platelets showed minimal levels on the thirteenth day. Recovery to levels above 100,000/cu.mm. was evident on the twenty-first day. In one patient, treated with marrow infusion, regeneration of platelets was observed on the sixteenth day. The author felt that autologous bone marrow administration played a minor if any role in the hematologic recovery of these patients.

In 1961, Smiley, Martin-Villar and Belanger treated 10 patients with a variety of malignant disorders with single intravenous injections of 0.4 mg. $\text{HN}_2/\text{kg. body weight}$.⁹ Five of the patients received 1.3 to 3.0 billion autologous bone marrow cells intravenously two to three hours after injection of medication. Marrow had been withdrawn shortly before treatment and preserved at 4° C. until returned to the subject. Viability of stored marrow was demonstrated by the ability of these cells to synthesize DNA, using tritiated thymidine. The authors found that there was no difference in depth or duration of neutropenia regardless of bone marrow transfusion. Fall in platelets was identical in both groups and recovery was delayed slightly in patients who received bone marrow suspensions. The question of whether there was any cytotoxic effect of HN_2 on infused bone marrow within two hours was discussed and considered unlikely.

In 1962 Cristoffanini reviewed current problems in "Transplantation of Bone Marrow" and stated, "Autologous bone marrow transplantation, either preserved or used after the marrow had stood for short periods of time has been successfully used as a method to raise the maximum tolerated dose of X-irradiation or of chemotherapeutic agents."¹⁰ He cited McFarland and Black as partial basis for this statement. He presented a figure, presumably from his own experience, since this case does not appear in any of the cited literature. This was a patient with carcinoma of lung treated with 1.1 mg. $\text{HN}_2/\text{kg. body weight}$ who received autologous bone marrow containing 7.0 billion nucleated cells after chemotherapy. Leukocytes fell to extremely low levels on the eighth day and began to rise six days later. Platelets fell at about the same time but showed an increase five days later, reaching levels of 600,000 to 700,000/cu.mm. about one month after chemotherapy.

In the present study patients with a variety of malignant disorders, mainly in lymphoma and pulmonary groups, were treated with 0.2 and 0.3 mg. $\text{HN}_2/\text{kg. body weight}$ on each of four consecutive days. Where autologous bone marrow transplantation was contemplated, marrow was removed from posterior iliac crests and/or sternum, in some instances tagged *in vivo* or *in vitro* with tritium labeled thymidine, glycerol added to 15 per cent volume, frozen at 1° C. per min. to -25° C., rapidly to -50° C., and stored at -80° C. in dry ice and alcohol. Marrow was reinfused into some patients 6 to 12 days after completion of HN_2 infusion. This was accomplished by diluting 10 ml. samples stepwise with 35 per cent and 5 per cent glucose solutions to a final volume of 50 ml. and a glycerol concentration of 3 per cent. Injection was through rubber tubing of a continuous infusion in the antecubital vein. Total nucleated cell counts were made before freezing and after thawing. When administered they ranged between 4.0 and 9.0 billion per treatment. Following the latter procedure only mild or no febrile reactions occurred, and hemoglobinemia and hemoglobinuria were insignificant and transient. Radioautographs and routine films of marrow suspension before freezing and after thawing suggested that nucleated cells and mature erythrocytes were extraordinarily well preserved and viable. After the last HN_2 injection, complete blood studies including hemoglobin, red blood cells, white blood cells, platelets, reticulocytes, hematocrit, and differential counts were performed daily. In those patients receiving autologous bone marrow, concentrates of peripheral leukocytes and bone marrow aspirations were made daily for radioautograph examination of samples. Cultures of bone marrow before freezing and after thawing were all sterile.

There were nine patients in the first group, each of whom received a total dose of 0.8 mg. HN_2/kg . body weight. Bone marrow was reinfused in five cases between the 6th and 12th days after the last HN_2 injection. This usually coincided with lowest level of leukocytes and platelets. In the treated group white cells began to rise on the 20th to 29th day, and platelets on the 13th to 23rd day after last HN_2 injection. Labeled cells were never recovered in preparations from peripheral blood or bone marrow. A typical case report follows:

Patient W., adult male, age 60 years, was treated for inoperable carcinoma of lung (FIGURE 1). He received intensive radiation therapy to chest prior to chemotherapy. Bone marrow was labeled *in vivo* during the 24-hour period prior to aspiration, using 20 m.c. of tritium labeled thymidine. Leukocytes fell below 5,000/cu.mm. six days and platelets below 100,000/cu.mm. nine days after therapy. Tagged bone marrow was reinfused on the 12th day, at which time leukocytes were 1,500/cu.mm. and platelets 28,000/cu.mm. Platelets rose above 100,000/cu.mm. 19 days, and white cells above 5,000/cu.mm. 21 days after last HN_2 injection. Thrombocytes ultimately reached 854,000/cu.mm. 22 days after completion of chemotherapy. Radioautographs of bone marrow and leukocytes in peripheral blood were entirely negative.

In subjects treated with 0.2 mg. HN_2/kg . body weight on four consecutive days who received no marrow, leukocytes fell below 5,000/cu.mm. in two to four days, and platelets under 100,000/cu.mm. in 6 to 12 days after treatment. Recovery of leukocytes beyond 5,000/cu.mm. mark was observed between the 18th and 23rd days. Platelets reached the 100,000/cu.mm. level on the fifteenth to nineteenth day. Normal values for leukocytes and platelets were observed earlier in untreated patients. Hematologic data of one of these three cases are shown in FIGURE 2:

Patient WP, male, age 38 years, was treated for inoperable carcinoma of lung with 0.2 mg. HN_2/kg . body weight on four consecutive days. Leukocytes fell below 5,000/cu.mm. and platelets below 100,000/cu.mm. on the third and sixth days after last injection, respectively. No bone marrow was reinfused and leukocytes rose above 5,000/cu.mm. and platelets above 100,000/cu.mm. on the twenty-fifth and fifteenth days, respectively.

In the last group four patients were treated with 0.3 mg. HN_2/kg . body weight on four consecutive days. None of these patients received chemotherapy of any kind prior to the present course. Leukocytes fell below 5,000/cu.mm. in one to three days and platelets below 100,000/cu.mm. in six to nine days after last HN_2 injection. In the recovery phase minimal normal levels were attained in 21 to 34 days for leukocytes and 18 to 19 days for platelets. An example of one such patient is shown in FIGURE 3:

Mrs. Ht, female, age 60 years, was treated for diffuse reticulum cell sarcoma with 0.3 mg. HN_2/kg . body weight on four consecutive days. White blood cells fell below 5,000/cu.mm. and platelets below 100,000/cu.mm. on the third and ninth days after the last injection, respectively. Leukocytes were depressed to 1,000/cu.mm. and platelets to 20,000/cu.mm., rising to normal levels on the twenty-first and eighteenth days, respectively. Rebound phenomena in white cells and platelets are clearly seen.

Superimposition of results of leukocyte and platelet counts in the entire group of untreated patients, which includes those receiving total doses of 0.8 and 1.2 mg./kg. shows a striking similarity (FIGURE 4).

A summary of changes in leukocyte levels in peripheral blood in all three groups is presented in FIGURE 5: Data on patients receiving 0.2 mg./kg. and autologous marrow reinfusion at time of maximal depression of white blood cells are shown in the upper part, those receiving same dose without bone marrow infusion are presented in the middle portion, and in the lower third are data on subjects who received 0.3 mg./kg. on four consecutive days. As previously noted reduction in leukocytes in the 0.3 mg./kg. group who received no marrow is essentially the same as in the 0.2 mg. patients. The recovery phase is slightly delayed in two patients in the 0.3 mg. group, but one patient showed a rise which compared favorably with any of the 0.2 mg. subjects. Differences were even less marked in the analysis of platelet changes shown in FIGURE 6. No perceptible difference is seen. It is noteworthy that in all three groups there is a rebound in total number of leukocytes and platelets in the recovery phase, more prominently in the latter.

It would therefore appear that following administration of 0.8 or 1.2 mg. HN_2 /kg. body weight there is no significant difference in the rate of fall or return to normal of leukocytes and platelets, regardless of reinfusion of autologous bone marrow at the time of maximal leukopenia and thrombopenia. Comparing these results with those of previously cited workers who reinfused autologous bone marrow within 24 hours after HN_2 injection it appears that there was probably no protection afforded, as leukopenia and thrombopenia developed and recovery from these states occurred at about the same time in treated and untreated patients.

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- See Supply?*
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FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

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