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Myelograms Relating to Anemia and Hematopoiesis Following Thermal Injury in Rats*. (22988)

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Anemias following thermal injury in man and laboratory animals have been repeatedly observed(1-6) and have been shown in part to result from increased rate of hemolysis(1, 2,5) and disturbed liver function(4). Suppressed medullary erythropoiesis has also been suggested as contributing to the anemia by James and co-workers(2) while Moore and his co-workers(1) and Wintrobe and his co-workers(7) have presented data from radioiron uptake studies that indicate direct medullary inhibition. However, the work of Davis, Alpen and Davis(5) and Davis, Alpen and Sheline(6) showed an increase in rate of radioiron uptake which they interpreted as evidence for increased extramedullary and medullary erythropoiesis.

In this investigation we have studied the quantitative changes in femoral marrow of control rats, bled rats and thermally injured rats. We have attempted to discover from the data (a) whether suppression of the medullary hematopoiesis exists following thermal injury and (b) whether there is any quantitative difference in medullary hematopoiesis between moderately injured animals and severely injured animals.

Materials and methods. Unshaved male Wistar rats weighing between 200 and 210 g were used. Rats were housed individually in wire mesh cages at room temperature varying between 72 and 78°F and relative humidity varying between 20 and 40%. Except for the immediate 10 hour period following injury, all rats received Purina Laboratory Chow *ad lib.* This diet was supplemented once a week with carrots. Groups A and B received thermal injuries of 50 ± 2% and 20 ± 2% of the body surface respectively. Group H was bled and infused. Group C was the uninjured control. Thermal injury was effected under ether anesthesia in water at 90°C for 35 seconds. Prior to experiment a polyethylene tube was inserted into the posterior facial vein of each rat. Immediately following the thermal injury each rat was infused with 4% body weight of 1/6 M sodium lactate solution and 14% body weight of 1.4% sodium chloride solution. The details of these procedures have been previously reported(8). Animals in control group H were operated, bled by cardiac puncture and infused as described above. Three cc of blood were withdrawn to approximate immediate blood loss from thermal injury. This volume was determined from our Fe^{59} studies. In each of the thermally injured groups A and B and in the bled group, H, 3 animals were sacrificed for femoral bone marrow studies at 24, 48, 96, 168 hours and 2 and 4 weeks following injury or bleeding re-

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spectively. Three animals were sacrificed at 8 weeks in groups B and H. The group A experiment was terminated at 4 weeks after injury due to difficulties of keeping such severely injured animals alive. In the uninjured control group C, 3 animals were sacrificed at each of the following intervals from initial starting date of experiment: 1, 4, and 8 weeks. Our method of bone marrow studies was in part suggested from experiments of Fruhman and Gordon(9). The rats were killed with ether and both femurs immediately removed and trimmed clean of surrounding tissue. Each femur was weighed before and after demedullation and average weight differences in mg were recorded as marrow weight. After initial weighing both femurs were carefully cracked and samples of marrow were drawn into certified erythrocyte pipettes to the 0.2 level. Six such pipettes were prepared. To 3 of these Hayem's solution was added and to the other 3 the standard acetic acid-gentian violet white cell diluent was added. The diluent in all pipettes was added to the 101 mark. After the pipettes were shaken for 3 minutes, certified hemacytometers were charged. Cells were counted in the 4 corner and center squares of the centrally ruled 1 mm square. The average of 6 counts, 2 from each pipette, was determined for total leukocyte and erythrocyte values recorded. Immediately after taking the marrow samples, several marrow smears were made by the imprint smear technic. These smears were fixed with osmic acid vapor and stained with Wright-Giemsa. From the smear preparations, 1000 leukocytes were identified and classified based upon the descriptions given by Endicott and Ott(10) into the following classes: a. immature neutrophilic granulocytes; b. mature neutrophilic granulocytes; c. lymphocytes; and d. other cells. Each class of cells was expressed in terms of cells per mm^3 by multiplying the total leukocyte count by the decimal fraction of each particular class.

Results. Marrow weights. Average marrow weights for all groups are shown in Fig. 1. Uninjured controls, group C, showed progressive increase with age. In bled group H,

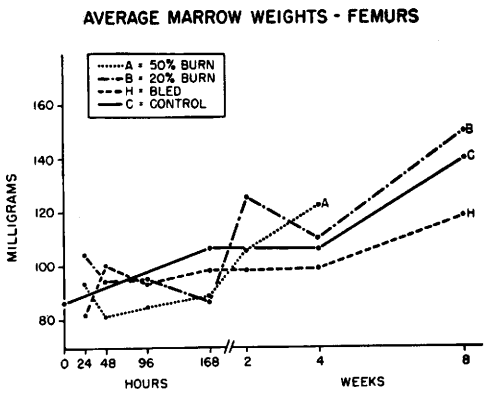

FIG. 1. Avg wt of marrow from right and left femurs in mg determined from differences in femur wt before and after demedullation. Each point on each curve represents avg values for 3 rats.

a sharp increase occurred from 24 to 48 hours after bleeding followed by a slightly lower value at 96 hours. From 96 hours after bleeding a progressive increase was observed. The animals given 50% thermal injuries showed a decrease at 48 hours after injury and then a progressive increase. The animals given 20% thermal injuries showed increased marrow weights at 24 hours after injury which was followed by a progressive depression throughout the first week. Then there occurred a sharp increase at 2 weeks after injury. Subsequent values paralleled those of the uninjured controls. *Erythrocytes.* Average erythrocyte counts are shown in Fig. 2. In uninjured controls there occurred an increase of erythrocytes with age. Bled con-

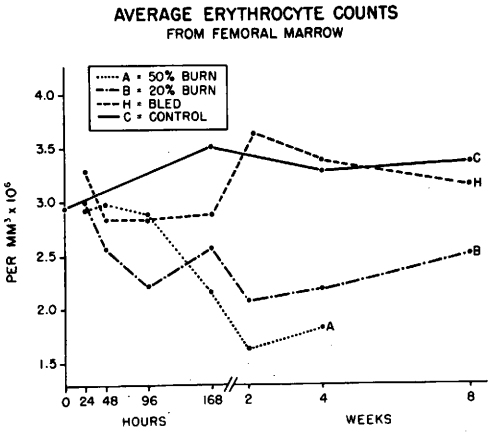

FIG. 2. Determined from hemacytometer counts of diluted femoral marrow. Each point on each curve represents avg values for 3 rats.

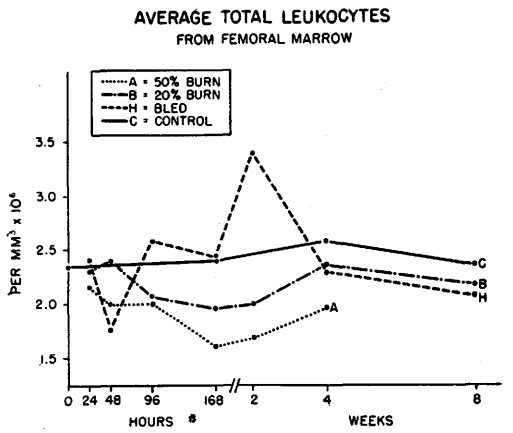

FIG. 3. Determined from hemacytometer counts of diluted femoral marrow. Each point on each curve represents avg values for 3 rats.

trols showed a decrease in erythrocytes during the first week after bleeding following which their erythrocyte numbers were not significantly different from uninjured controls. The animals given 20% thermal injuries showed a progressive depression through the first 96 hours, a recovery to the 48 hour level at 1 week after injury, then a secondary depression at 2 weeks resulting in numbers below the 96 hour post-injury level. From 2 weeks through 8 weeks their erythrocyte level progressively increased toward the pre-injury level. The animals given 50% thermal injuries showed a precipitous decline in erythrocyte numbers from 96 hours through 2 weeks after injury and at the 2 week interval reached a level approximately 400,000 cells less than the corresponding value for the animals given 20% injuries. At 4 weeks after injury the erythrocyte numbers of the animals given 50% thermal injuries increased by approximately 150,000 cells.

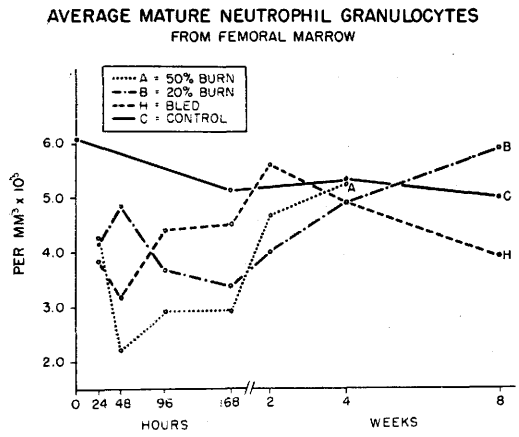

FIG. 4. Determined from smears by multiplying total leukocyte counts from femoral marrow by decimal fraction of these cells out of 1000 classified leukocytes. Each point on each curve represents avg values for 3 rats.

Leukocytes. Since the changes in the immature neutrophilic (n.) granulocytes were found to parallel the changes in the total leukocytes, (Fig. 3) the former have not been presented here. The uninjured control animals, group C, maintained approximately the initial levels of total leukocytes and of mature n. granulocytes, (Fig. 4), throughout the experimental period. The bled animals showed a depression of the numbers of total leukocytes and mature n. granulocytes during the first 48 hours after bleeding following which they progressively recovered and attained levels well above the controls by the second week. From the fourth week through the eighth week after bleeding the numbers of these cells approximated those of the control group C. In the animals given 20% thermal injuries the total leukocytes and mature n. granulocytes decreased from 48 hours through 1 week following injury. Then they increased through 4 weeks after which the total leukocytes leveled off parallelling the uninjured control and bled groups. The mature n. granulocyte numbers of the 20% injured animals were greater than those of the uninjured controls at 8 weeks following injury. The 50% thermally injured group showed a precipitous reduction in the numbers of mature n. granulocytes during the first 48 hours after injury. Then their numbers increased through 4 weeks at which time they reached the control level. The total leukocytes of the 50% injured group declined through the first week following injury. At 4 weeks the numbers of these cells in this group were considerably less than those in any of the other groups.

Discussion. The thermally injured rats studied in this investigation differ from similarly injured patients in the therapeutic procedures necessary for survival. Following the primary shock period no intravenous

fluids were given and no antibiotics were administered. The skin over the burned area remained in place for 3 to 4 weeks following the injury. The area under the injury became hard and dry within 24 to 48 hours following sloughing. Thus in our judgment, the quantitative changes observed in the bone marrow, reflecting either a stimulative or a suppressive effect, would be uncomplicated by the excessive loss of fluid and blood cells from granulating wounds, or excision and grafting procedures.

Our data show a clear-cut difference in the marrow erythrocyte populations between the burned rats and the bled and uninjured controls. The bled animals after the first week following bleeding showed a definite marrow stimulation which resulted in erythrocyte levels comparable to the uninjured controls. Both thermally injured groups of animals showed decreased numbers of erythrocytes following the first week after injury which we interpret as a suppression of marrow function. This interpretation is strengthened by our observations of erythrocyte counts in the peripheral circulation of similarly injured animals(11). We found an approximate reticulocyte increase of only 3% above the preinjury level at 2 weeks after injury. In addition no significant increase in the number of nucleated erythrocytes was found. The reduction in marrow erythrocyte numbers in the injured animals was directly related to the severity of the injury. Following the 1 week post-injury interval, the numbers of marrow erythrocytes were significantly lower in the animals given 50% body surface thermal injury. Further evidence of a suppression of medullary hematopoiesis was found when the total leukocyte counts of the bled and injured rats were compared. From the first through the second week after bleeding a tremendous increase in the medullary total leukocytes occurred while only a very slight increase in the numbers of these cells occurred in the two thermally injured groups. Also it was found that the numbers of the medullary total leukocytes in the injured animals were lower in the more severely injured. The numbers of the medullary immature granulocytes which are a measure of the hematopoietic activity were observed to be suppressed from 96 hours through 2 weeks after injury when compared to those at similar intervals in the bled animals. Again it was found that the numbers of n. granulocytes were significantly lower in the more severely injured. Thus our data support the conclusions drawn from radioiron uptake studies by Moore *et al.*(1) and Wintrobe *et al.*(7) that suppression of marrow function is definitely a contributing cause of anemia after thermal injury. Our data would not support the conclusions of Davis *et al.*(5,6) drawn from radioiron uptake studies in thermally injured rats that increased medullary hematopoiesis occurs following injury.

Summary. Marrow weights and the numbers of cells per mm^3 in femoral marrow of erythrocytes, total leukocytes, immature and mature n. granulocytes were obtained in uninjured rats, bled rats, and rats given thermal injuries of 20% and 50% body surface. Significantly lower numbers of erythrocytes, total leukocytes and n. granulocytes were found in the thermally injured rats during first two weeks following injury compared to the numbers of these cells occurring at similar intervals in the uninjured controls and bled animals. The numbers of these cells following thermal injury were found to be inversely related to the percentage of body surface injured. These data support the conclusion that a suppressed medullary hematopoiesis occurs after thermal injury and is partially responsible for the circulatory anemia found in thermally injured animals.

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Effect of Temperature on Ionographic Mobilities in Paper Stabilized Media.* (22989)

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In a recent article on paper electrophoresis (1), Forbes and Taylor reported that although the rate of electromigration of beta lipoproteins was considerably less at 5°C than at room temperature or at 35°C, no effect of temperature was evident in lipoproteins migrating with the albumin, alpha$_1$ globulin. They also reported that rate of movement of the main serum components was apparently the same at different temperatures. The non-uniformity of the effect of temperature on mobilities of the substances studied by them was in such marked contrast to those observed in this laboratory(2,3) that it was decided to reinvestigate the effect of temperature on ionographic mobilities in paper stabilized media for a range of substances varying from low molecular weight materials, such as a simple dye, to more complex substances such as plasma proteins and lipoproteins.

Methods and material. The Precision Ionograph,§ which utilizes the horizontal paper strip in a water-saturated atmosphere(4), was used with Whatman No. 1 filter paper strips (0.5 inch in width and 40-45 cm in length) and a veronal buffer of pH 8.6 and ionic strength of 0.03 and 0.01. The apparatus permits use of 7 paper strips simultaneously. In nearly all cases, the runs on each migrant were made at 4 different temperatures, namely 25°C, 15°C, 9°C and 4°C. During each run the temperature was maintained at $\pm$ 0.2°C by circulating liquid of constant temperature through the double-walled shell of the ionographic apparatus. The substances whose mobilities were determined included bromphenol blue (Hartman-Leddon Co., Philadelphia), crystalline bovine plasma albumin (Armour Laboratories, Chicago), and the following serum fractions, obtained from fresh pooled human serum, namely, albumin, alpha lipoprotein, alpha globulin, beta lipoprotein and gamma globulin. The veronal buffer having an ionic strength of 0.01 was employed for the bromphenol blue and bovine plasma albumin, while an ionic strength of 0.03 was used for all determinations on human serum fractions. The average time of a run was 3-4 hours, and a potential gradient of 4-5 volts/cm was used, maintained constant during the run. For the runs at 25°C helium was introduced into the water-saturated atmosphere while at lower temperatures, the water-saturated atmosphere alone was employed. Equilibration time, *i.e.*, the time the paper strips were permitted to stand, after wetting and with the current on, to reach a constant value for the ratio of "weight of buffer solution" to "weight of paper" was determined for the particular paper and buffer used, before beginning the runs.

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‡ Standard Oil Foundation Research Fellow; supported by Standard Oil Co. (Ind.).

§ Precision Scientific Co., Chicago, Ill.