

Hemopoiesis in pregnant beagles following low-dose total-body irradiation and surgery^{1,2}

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Summary. Hemopoietic perturbations were observed in gravid beagles subjected to whole-body ionizing radiation during midpregnancy followed by the additional trauma of surgery, with alterations in peripheral blood hemogram parameters, bone marrow morphology, and concentration of marrow-derived granulocytic- and erythrocytic-committed stem cells. However, the trauma of surgery combined with irradiation had no apparent effects on the contralateral uterine horn with the remaining pups, with no indications of miscarriage, absorption of pups, or other complications of pregnancy.

Studies were conducted with 5 groups of beagles to evaluate hemopoietic changes during pregnancy in large-sized laboratory experimental animals: 1. control, 2. pregnant, 3. pregnant+surgery, 4. pregnant+irradiated and 5. pregnant+irradiated+surgery. Complications to the contralateral uterine horn with remaining pups were assessed. The peripheral blood hemogram parameters, bone marrow morphology, and hemopoietic committed stem cells [granulocyte-macrophage colony-forming cell (GM-CFC), erythroid burst-forming unit (BFU-E), and erythroid colony-forming unit (CFU-E)] were monitored with clonogenic in vitro assays at selected times during the course of pregnancy.

Materials and methods. Healthy normal and gravid beagles, with confirmed dates of conception, were obtained from Hazleton Research Animals Inc., Hazleton, VA, and the Springville Laboratory of Roswell Park Memorial Institute, Buffalo, N.Y. Dogs were housed individually in stainless steel cages in temperature-controlled rooms with a 12-h light-dark cycle. They were maintained on kibbled laboratory dog food and water ad libitum, supplemented once a week with a high-protein canned-meat ration. All dogs received continuous attention from the Veterinary Department staff.

At midpregnancy (day 33), dogs were placed in a plastic holding cage. They received a total-body irradiation (TBI) exposure of 90 rad from the bilateral AFRRRI ⁶⁰Co source, at a rate of 40 rad per min. Subsequently, on different days of pregnancy, peripheral blood was collected from the cephalic vein for determination of red blood cell counts (RBC), white blood cell counts (WBC), hemoglobin (Hgb) concentration per dl (g/dl), and hematocrit (Hct) percent values. Bone marrow aspirates were taken from the ribs of anesthetized dogs immediately before surgery. At selected days of pregnancy, nonirradiated and irradiated pregnant dogs were anesthetized, a ventral midline incision was made on each occasion, and 1 horn of the uterus with pups was removed. Pregnant dogs were allowed to recover from surgery for at least 5 days before removal of the other horn of uterus with the remaining pups of the litter. Bone

marrow aspirates and peripheral blood samples were obtained immediately before the 2nd surgical procedure. Bone marrow nucleated cells were separated from the whole aspirate by gradient sedimentation with Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) followed by washing with McCoy's 5a medium containing 10% fetal calf serum. Cytospin smears were prepared and stained with benzidine and counter-stained with Wrights and Giemsa blood stain solutions for evaluation. The maternal marrow cells were cultured in soft agar cultures as routinely done in our laboratory using 15% (v/v) plasma from endotoxin-stimulated dogs as the source of colony-stimulating activity (CSA) for 2×10⁵ nucleated cells per dish³. After 10 days of incubation, colonies of 50 cells or more were counted and considered to be derived from GM-CFC. Microplasma clot cultures were established, as previously outlined in detail⁴, to study both erythroid committed stem cells: BFU-E and CFU-E. Cultures plated with 50,000 nucleated cells per 0.1 ml and 0.05 unit of erythropoietin per 0.1 ml (EPO, a protein extract from anemic sheep plasma, Step III, 6.7 units per mg protein, lot No.3023-3, Connaught Labs, Inc., Swiftwater, PA) were harvested after 72 h of incubation at 37 °C with humidified 4% CO₂ in air. Colonies of 8 or more benzidine-stained positive cells were counted as representative of the more mature erythroid progenitor cell, CFU-E. Clots with 0.3 unit of EPO per 0.1 ml were harvested at 9 days of culture. Clusters of benzidine-stained positive cells were counted as representative of the younger erythroid progenitor cell, BFU-E.

Results. Peripheral blood hemogram. The peripheral blood erythron hemogram values [RBC counts, Hct percents, and Hgb concentrations] of an initial and subsequent samplings during pregnancy of normal pregnant, irradiated pregnant and similar groups subjected to the additional trauma of surgery were 55% below values for normal nongravid beagles⁵ (RBC 7.82×10⁶/mm³, Hct 48.5%, Hgb 16.7 g/dl). By day 43 of pregnancy, white blood cell counts of all nonirradiated pregnant dogs were above the normal range for nongravid female dogs⁵ (fig. a). However, all irradiated

Table 1. Normal and pregnant (P) beagle bone marrow blood cell distribution^a

	n	Granulocytic Proliferative ^b	Nonproliferative ^c	Nucleated erythroid	Monocyte and macrophage	Lymphocyte	Plasma cell
Normal	8	4.5±1.3	55.6± 6.9	36.0± 8.5	0.5±0.5	3.5±0.7	0.2±0.2
Normal P	5	13.9±1.9 ^d	49.4± 6.2	24.2± 7.1	0.6±0.6	11.4±2.4 ^d	0.9±0.3
Normal P, surgery	3	12.6±2.9 ^d	60.3± 0.3	11.3± 2.7 ^d	1.0±0	11.8±1.5 ^d	2.6±1.4
Irradiated P	3	5.0±1.9	53.0±10.0	29.0±12.0	0.2±0.2	11.4±2.4 ^d	1.1±0.6
Irradiated P, surgery	2	14.5±2.0 ^d	48.0± 7.0	25.0± 8.0	0.5±0.5	10.0±4.5	1.0±1.0

^aValues are expressed as mean percent ± SE. ^bGranulocyte proliferative cells include myeloblast, promyelocyte, and myelocyte. ^cGranulocyte nonproliferative cells include metamyelocyte, band, and mature granulocyte. ^dValues are significantly different (p<0.05) from normal beagle.

pregnant dogs (10 days after irradiation) had WBC values 70% below values for nongravid and nonirradiated pregnant dogs. The trauma of surgery in the irradiated pregnant dog (at 14–22 days postirradiation) resulted in a slight increase in WBC values but still below values of all nonirradiated pregnant dogs (fig. b), whereas surgery in nonirradiated dog resulted in a decrease in WBC values. Examination for peripheral blood smears indicated no significant differences from normal in the differential distribution of circulating blood cell elements.

Bone marrow hemogram. The differential distribution of bone marrow cells from normal and pregnant dogs (days 41–57) is reported in table 1. Significantly fewer proliferative granulocytic cells were seen (myeloblasts, promyelocytes, and myelocytes) in the irradiated pregnant dog than in the normal pregnant beagle ($p < 0.01$). Following surgery, a significant decrease was seen in marrow nucleated erythroid cells in the nonirradiated pregnant dog ($p < 0.05$) compared to the normal pregnant group. With the exception of the group of irradiated pregnant dogs, all pregnant dogs had significantly more bone marrow proliferative granulocytic cells than did the normal canine marrow ($p < 0.125$). More lymphocytes were found in the marrow of all pregnant beagles than in normal beagle marrow ($p < 0.01$).

Compared to normal canine marrow GM-CFC values (186.8 ± 10.0 per 10^5 cells), there was a 71% decrease in rib marrow-derived GM-CFC values of normal pregnant dogs that had undergone surgery earlier in the 2nd trimester. Irradiated pregnant dogs following surgery (at 14–22 days post-TBI) responded differently from nonirradiated dogs. Post-surgery irradiated GM-CFC values were only slightly different than before surgery, but were 138% higher than values for nonirradiated dogs following surgery. Although the trauma of surgery caused a decrease in nonirradiated pregnant marrow GM-CFC activity to values significantly below those for normal nongravid dogs, CFU-E were 48% higher than values for nongravid beagles (normal = 309.6 ± 25.0 per 10^5 cells).

At day 44 of pregnancy, an irradiated gravid beagle had 1.3 BFU-E per 10^5 bone marrow cells plated. The same dog at day 55 of pregnancy (22 days postirradiation and 11 days post-surgery) had 6.0 BFU-E per 10^5 bone marrow cells plated. Aggregates of 3 or 4 benzidine-stained positive cells were also observed in the 9-day microplasma clot cultures of some nonirradiated pregnant beagles, but they were not

as pronounced as those observed in the irradiated pregnant dog.

A comparative evaluation of peripheral blood percent RBC/WBC values is reported in table 2. The peripheral blood percent RBC/WBC values are lower in the pregnant beagle than in the normal beagle, which reflects pregnancy-induced anemia in the beagle. The stimulated bone marrow erythroid progenitor cell activity of the pregnant dog is also reflected by a higher percent CFU-E/GM-CFC value than those in normal beagles. These data suggest an enhanced marrow erythropoietic activity in the gravid beagle, probably at the expense of marrow granulocytopoiesis.

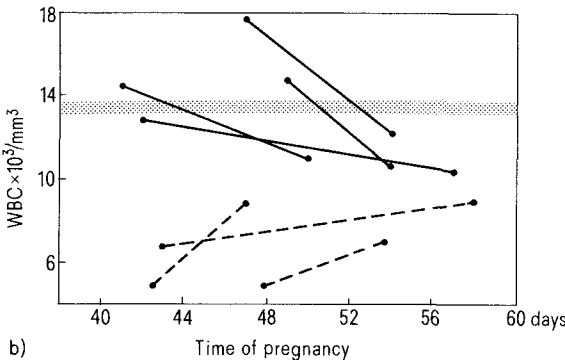
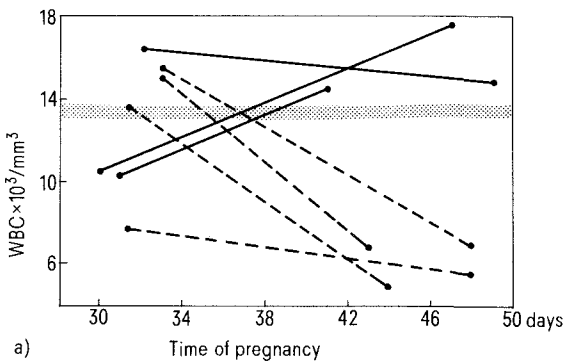
Discussion. These experiments were designed to use a large animal model for the purpose of studying a) alterations in hemopoiesis during normal pregnancy and following the trauma of total-body irradiation and surgery introduced during pregnancy and b) the effects on the contralateral uterine horn during the remainder of the pregnancy. Pregnancy-induced anemia in the beagle was documented as a decrease in all peripheral blood erythron indices (total RBC, Hct, and Hgb) with a concomitant increase in the peripheral blood WBC count. A leukocytosis during mid-pregnancy was also observed with rodents⁶. Perhaps the peripheral blood erythrocytopenia in the beagle served as the feedback regulator to the bone marrow to stimulate erythropoietic activity (i.e., to increase CFU-Es to above normal beagle values). Evaluation of the peripheral blood and the bone marrow following TBI to the pregnant dog during the midtrimester of pregnancy (day 33 of

Table 2. Comparison of erythropoietic and granulocytic activities in bone marrow and peripheral blood of pregnant beagle

	Pregnant	Irradiated-pregnant ^a	Post-surgery ^b Pregnant	Irradiated-pregnant
Percent values for bone marrow CFU-E/GM-CFC per 10^5 nucleated cells (normal beagle values = 145%–185%)				
Days 40–44	385	577	–	–
Days 47–50	100–325	–	517	–
Days 54–57	–	–	148–746	342
Percent values for peripheral blood RBC/WBC per mm^3 (normal beagle value = 59%)				
Days 30–33	32–58	–	–	–
Days 40–43	37–41	50	–	–
Days 47–50	30–31	54	31–46	32
Days 54–57	–	–	30–49	38

Peripheral blood samples and bone marrow aspirates were taken at selected subsequent times during gestation.

^aTBI on day 33 of gestation. ^bSurgery on day 41–49.



Pregnant beagle peripheral blood white blood cell counts. Solid line represents values for individual normal pregnant dogs. Broken line represents values for individual irradiated pregnant dogs (irradiated on day 33 of pregnancy). a Data for normal pregnant and irradiated pregnant beagles. b Data for normal pregnant and irradiated pregnant beagles subjected to surgery on day indicated by first time point for each dog. Normal beagle values: WBC $12.9 \times 10^3/\text{mm}^3$.

pregnancy) indicated a further decrease in peripheral blood erythron indices and a more profound reduction in peripheral blood leukocytes and marrow granulocytic proliferative cells compared to normal values. The trauma of surgery to nonirradiated beagles in midtrimester of pregnancy resulted in a greater decrease of all peripheral blood indices, marrow nucleated erythroid cells, and marrow GM-CFCs. The decrease in all the peripheral blood parameters of irradiated pregnant beagles (14–22 days post-TBI) following surgery may have stimulated erythroid progenitor cell values to increase.

The compensatory role of the pregnant beagle bone marrow to support enhanced erythropoiesis following the trauma of TBI and surgery is suggested by the increase in CFU-Es and BFU-Es, most likely at the expense of granulopoie-

sis. Most of the traumatized pregnant dog GM-CFC values were below the normal dog values, whereas all traumatized pregnant CFU-E values were above the normal dog values. It is evident that a dose of 90 rad gamma radiation and the additional stress of surgery has a dramatic and sustained effect on pregnant canine bone marrow hemopoiesis. Evidence is accumulating regarding myelopoietic responses following surgical or wound trauma postirradiation^{7–11}. Wiktor-Jedrzejczak¹² has recently reported enhanced hemopoietic stem cell activity in the postirradiated and bled animal. However, no apparent gross alterations were observed in uterine healing or in fetal growth in the contralateral uterine horn. In addition, no disruption in pregnancy was noted, however late the first surgery was performed.

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Change in the activation rate of voltage-dependent Ba^{2+} current by conditioning pre-depolarization

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Summary. When Ba ions replace Ca ions in the external solution, conditioning depolarizing voltage-clamp pulses slow the activation rate of the fast early inward currents in the ciliate *Stylonychia*, while inactivation is greatly reduced in the presence of Ba.

The voltage-dependent Ca channel has been shown to be permeable to Ba and Sr ions². In Ca-free Ba-solutions the early inward current is maintained at nearly its maximum amplitude during depolarizing pulses lasting for several hundred msec in many excitable cells, including ciliates^{3–7}. This appears to be due to the blockade of the K outward current^{7–9} and a reduction of inward current inactivation^{7,10}. The use of Ba as charge carrier through the Ca channel therefore provides a favorable opportunity to study the activation kinetics of this channel. In molluscan neurones^{3,11,12} and insect muscle¹³ the activation of Ba (and Ca) currents is accelerated with increasing membrane depolarization. I have studied how the activation kinetics of the Ca channel in the presence of Ba ions as charge carriers are affected by a prepulse. The time constant of activation of Ba inward currents is increased by 60% by a given depolarizing prepulse.

The experiments were carried out on the hypotrich ciliate *Stylonychia mytilus*. The membrane was voltage-clamped with the technique using 2 intracellular microelectrodes described previously⁶. The membrane potential was held at

–50 mV at its resting value. The membrane currents were not corrected for leakage current (< 1 nA) or capacitive currents, the time constant of the latter being smaller than 0.3 msec. The cells were first superfused in the standard Ca-solution containing 1 mM $CaCl_2$, 1 mM KCl and 1 mM Tris-Cl, pH 7.4. The test solution, containing 1 mM $BaCl_2$ instead of $CaCl_2$, was introduced while the cells were held in voltage-clamp to prevent repetitive excitation^{6,7}. All experiments were performed at $17 \pm 1^\circ C$.

The figure shows 4 sample recordings of Ba inward currents following +20 mV (to –30 mV) voltage-clamp pulses. In the figure, A, a depolarizing pulse of 160 msec duration elicited an inward current, which achieved a maximum of 11 nA within 5 msec, and decayed to a near steady-state of 9 nA. Thus, there is relatively little inactivation of this net inward current in Ba as compared to inward currents in Ca⁶. When 2 voltage pulses were given (fig., B), the 2nd pulse evoked a current with a much slower rate of activation. The peak amplitude of the current following the 2nd-step pulse eventually achieved about 85% of the control current, but only within 20 to 25 msec (with an interval of