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Fetal murine hemopoiesis following *in utero* low-dose irradiation

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SUMMARY

The influence of *in utero* low-dose ionizing radiation exposure on murine hemopoietic embryogenesis was investigated. *In vitro* assays such as micro plasma-clot cultures and double-layer soft agar cultures served as sensitive biodosimeters to determine erythropoietic and granulopoietic injuries. Day-10·5, HA/ICR, pregnant mice were irradiated with 0, 50, 100, 150, 200, or 300 rads, and day-14·5 fetal livers were studied for colony-forming unit-erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), granulocyte-macrophage colony-forming cell (GM-CFC), and macrophage-colony-forming cell (M-CFC) activity. Fetuses subjected to doses of 200 rads or higher on day 10·5 of gestation responded with a decrease in day-14·5 liver cellularity, reflecting injury to the developing organ and its inability to recover to the nonirradiated values. Difference in response between erythropoietin(EPO)-dependent and EPO-independent CFU-E strongly suggests existence of two populations of erythroid progenitor cells with different radiosensitivities. A dose of 200 rads markedly reduced CFU-E recovery, and a dose of 100 rads was sufficient to reduce BFU-E recovery to almost 10% of 0-rad values. Nonirradiated day-14·5 fetal liver had more GM-CFC compared to any of the irradiated fetuses, and a dramatically reduced M-CFC recovery occurred with each increase in dose following 150 rads. Our results showed that (1) fetal liver granulopoiesis is more sensitive to radiation injury compared to erythropoiesis, and (2) fetal liver has a greater potential for erythropoiesis recovery.

INTRODUCTION

Influence of *in utero* low-dose irradiation to developing embryos varies, depending on stage of development and exposure administered. Rugh (1971) described in great detail the comparison of radiation-induced congenital anomalies in mice and man, and suggested that rodent data may be extrapolated to man when embryologically comparable gestation ages are studied. Brent (1971) reviewed the literature dealing with variations in exposure rate of low-dose irradiation administered to rodent and human embryos and the resulting malformations and retardation effects. Studies with rats by Brent (1971) and Martin (1973), mice by Rugh (1971), and humans by Tubaina (1979) have

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shown that development of the fetus can be divided into three phases of radiation sensitivity: (1) exposure during the predifferentiation period results in reduced litter size because of effect on embryo implantation and fetoplacenta support; (2) exposure during active organogenesis may involve the most comprehensive injury involving defects of the nervous system, limb formation, organ differentiation, hematopoietic embryogenesis, and immunogenesis; and (3) exposure during the third stage of gestation of rapid growth may result in permanent retardation of overall growth, organ development, and brain development. However, Martin (1973) described the effect of prenatal irradiation on neonatal organ growth by monitoring changes in organ weights (i.e. liver, kidney, thymus, and spleen). It is generally agreed that the most critical period in the developing mammalian fetus for vulnerability to radiation-induced congenital defects is the second trimester of gestation during organogenesis (i.e. day 8 to 14 in the mouse and day 20 to 45 for man). It is at this decisive time that hematopoietic embryogenesis is actively occurring in the extraembryonic loci (i.e. the yolk sac) and commencing in the fetal liver. There is a wealth of experimental evidence that supports the migration hypothesis of the movement of stem cells from the yolk sac to the fetal liver *via* the peripheral blood (Barker, 1970; Djaldetti, Goldman & Fishman, 1978; Johnson & Moore, 1975; Metcalf & Moore, 1971; Pozzi, Andreozzi & Silini, 1972; Yoffey, 1971). The aim of the present study was twofold: to determine the effect of *in utero* low-dose gamma irradiation on fetal hepatic hematopoiesis, and to explore the use of *in vitro* assays to serve as sensitive biodosimeters of hematopoietic injuries. Fetuses were irradiated on day 10·5 of gestation, which is at peak activity for stem cells in the yolk sac (Metcalf & Moore, 1971), and fetal livers were studied on day 14·5 of gestation, when more than 60% of the cells are hematopoietically active (Metcalf & Moore, 1971; Pozzi *et al.* (1972).

MATERIALS AND METHODS

Mice

Virgin HA/ICR mice, 10–18 weeks old (Cumberland View Farms, Clinton, Tennessee), were randomly mated during a period of 24 h, designated as day 0 of gestation. All animals were maintained on a diet of Wayne Lab-Blox and acidified water (pH 2·5) and housed in a facility with a 12-h light–dark cycle. All mice were screened for murine pneumonia complex and *Pseudomonas* spp before each study.

Cell suspensions

Nontreated and irradiated 14·5-day-old fetuses were aseptically separated from maternal and extraembryonic connective tissues and washed in chilled physiological saline solution. For each dose point studied, at least 21 livers from three

litters were pooled in 5·0 ml chilled SAM* with 2% fetal bovine serum (heat-inactivated at 56 °C for 30 min). A single cell suspension was prepared using mechanical manipulations by flushing the tissue through a 5-ml pipette several times and then passing the crude preparation through needles of decreasing diameters (outlined by Weinberg & Stohlman, 1976). A sample was removed for morphological study and a cell count. Data reported per dose point represent the results from four separate matings and irradiation schedules.

In vitro *assays*

Micro plasma-clot cultures were used to assess fetal liver erythroid progenitor cell activity. One-ml cultures consisted of 0·1 ml cell suspensions (25000 nucleated cells), 0·3 ml fetal bovine serum (heat-inactivated; Rehautin F.S. Reheis Chemical Co., Illinois, Lot no. T46601 or Flow Laboratories Inc., Virginia, Lot no. 29101190), 0·1 ml beef embryo extract (McLeod, Shreeve & Axelrad 1974), 0·1 ml 10% bovine serum albumin (McLeod *et al.* 1974), 0·1 ml L-asparagine (final concentration 0·02 mg per ml, McLeod *et al.* 1974), 0·1 ml 10^{-4} M-2-mercaptoethanol, and 0·1 ml erythropoietin (EPO). Anemic sheep plasma (Step III, Connaught Laboratories Inc., Swiftwater, Pennsylvania, Lot no. 3023–3, 6·7 units per mg protein) served as the source of EPO and was added to the cultures for final concentrations of 0·125 units per ml for cell-forming unit-erythroid (CFU-E) and 3·0 units per ml for burst-forming unit-erythroid (BFU-E). For control cultures, SAM was added instead of EPO. Each ingredient was either reconstituted or diluted with SAM. All ingredients were maintained on ice and mixed with 0·1 ml bovine citrated plasma (GIBCO), maintained at 37 °C, immediately before plating in microtiter wells (sterilized by UV light for 3 h, Cooke Engineering Co., Virginia). For each experimental group, six 0·1-ml microtiter well cultures were established and incubated at 37°C with humidified, 5% CO_2 in air. Three-day cultures were harvested, fixed, stained with benzidine and Giemsa, and examined for aggregates of eight or more benzidine-positive cells, reflecting CFU-E. Cultures harvested on day 9 with benzidine-stained positive aggregates of more than 100 cells or several clusters of cells reflected the younger erythroid progenitor cell BFU-E.

The technique used by MacVittie (1979) for the double-layer soft agar cultures was used to assess granulocyte-macrophage colony-forming cell (GM-CFC) activity of fetal liver cells after 8 days of incubation and macrophage-colony-

* SAM is Supplemented Alpha Modification of Eagle's Medium (Frank Monette, personal communication): 10·075 g Alpha Medium (Flow Laboratories, Inc., Virginia), 10 ml non-essential amino acid solution (10 mM, 100X concentration, Grand Island Biological Company, New York), 10 ml sodium pyruvate solution (100 mM, 100X concentration, GIBCO), 10 ml L-glutamine (200 mM, 100X concentration, GIBCO), 20 ml penicillin (5000 units)-streptomycin sulphate solution (5000 mg) (Flow Laboratories), 1·87 g sodium bicarbonate powder, and 950 ml tissue culture water (DIFCO Laboratories, Inc., Michigan). Final pH was adjusted to 7·5 with NaOH. The 1000 ml of medium was prepared, millipore-filtered, aliquoted into 100-ml volumes, and stored in the refrigerator for no longer than 3 weeks.

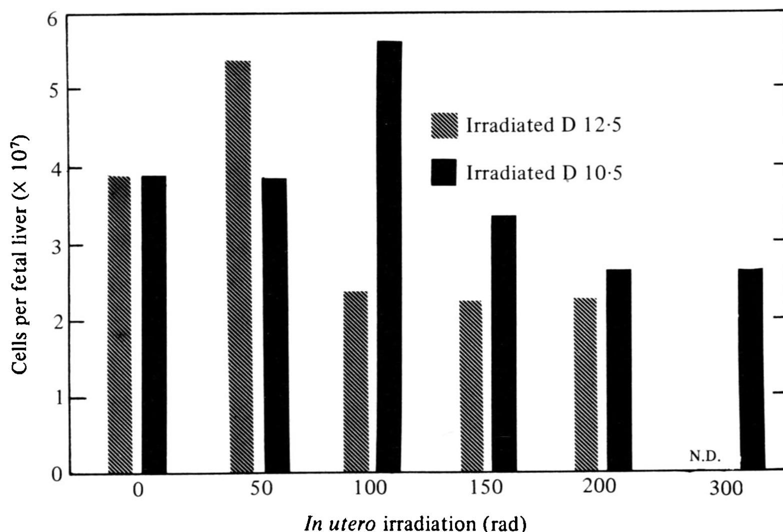

Fig. 1. Effect of low-dose irradiation on HA/ICR day-14·5 fetal liver cellularity. Fetuses were irradiated *in utero* on day 10·5 or 12·5 of gestation. N.D. = not determined.

forming cell (M-CFC) activity after 25 days of culture. Pregnant mouse uteri extract (PMUE) was used as the source of colony-stimulating activity (CSA) for $5{\cdot}0 \times 10^4$ nucleated day-14·5 fetal liver cells plated per ml in all the agar cultures.

RESULTS

In utero low-dose irradiation of 50 to 200 rads on day 10·5 or day 12·5 of gestation had no visible effects on litter size or viability of day-14·5 fetuses (i.e. 8 pups per litter). However, 300 rads on day 10·5 of gestation appeared to cause a decrease in number of viable day-14·5 fetuses per litter (i.e. about 39% of fetuses were observed to be aborted when the intact uterus was removed). Day-14·5 HA/ICR-fetal liver cellularity was $3{\cdot}9 \pm 0{\cdot}9 \times 10^7$ nucleated cells (mean ± S.E.M.), of which 36·5% were proerythroblasts and basophilic erythroblasts, 46·5% polychromatophilic erythroblasts, 6·5% myeloblasts and myelocytes and 8·0% mononuclear cells.

Cellularity

Differences in values obtained for day-14·5 fetal liver cellularity after *in utero* irradiation on day 10·5 of gestation and on day 12·5 of gestation are shown in Fig. 1. The two values for each dose point represent 4 days of recovery activity from day 10·5 to day 14·5 and 2 days activity from day 12·5 to day 14·5. Whereas 50 rads administered on day 12·5 caused a 39% increase in tissue cellularity compared to 0-rad values, no change in cellularity occurred when fetuses were

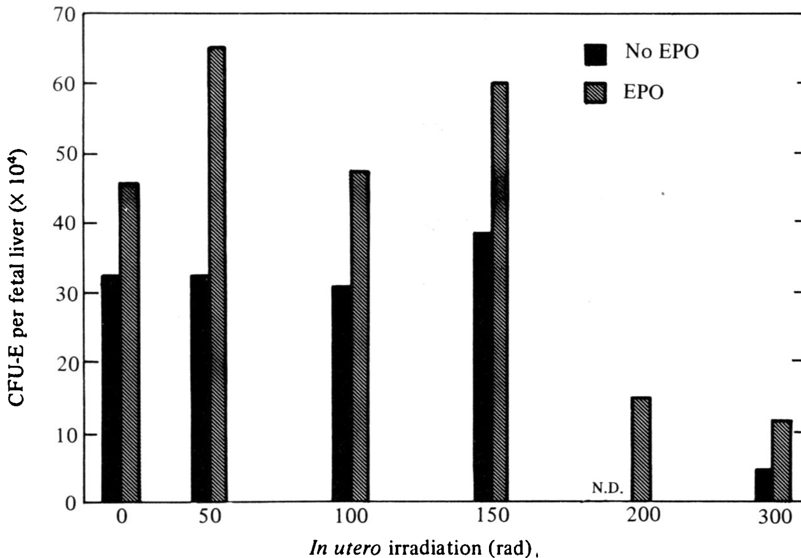

Fig. 2. Effect of low-dose irradiation on CFU-E per day-14·5 fetal liver. HA/ICR fetuses were irradiated *in utero* on day 10·5 of gestation. Mean values of CFU-E in plasma clot cultures containing 25000 cells per 0·1 ml and 0·125 units of erythropoietin (EPO) per ml are compared to spontaneous colony formation. N.D. = not determined.

irradiated on day 10·5. Commencing with the 100-rad dose and maintained with 150 and 200 rads, day 14·5 tissue cellularity of fetuses exposed on day 12·5 were decreased to 59 % of control values, demonstrating radiation injury. Growth of fetal liver following irradiation was shown to be further reduced during day 10·5 to day 14·5 of gestation with the observed decrease in tissue cellularity values.

Fetal liver erythropoiesis

The effect of low-dose irradiation to day-10·5 fetuses on day-14·5 hepatic erythropoietic activity is shown in Fig. 2. Doses of 50 and 100 rads had no effect on the EPO-independent CFU-E, and 150 rads slightly increased the values. In contrast, EPO-dependent CFU-E values increased significantly ($P = 0{\cdot}05$) following 50 and 150 rads and moderately after 100 rads. Doses of 200 and 300 rads markedly reduced all CFU-E values ($P = 0{\cdot}0125$). The differences in response between the EPO-dependent and EPO-independent CFU-E strongly suggests the existence of two populations of erythroid progenitor cells with different radiosensitivities.

Cultures plated with 3·0 units EPO per ml and incubated for 9 days reflected fetal hepatic BFU-E activity of irradiated fetuses (Fig. 3). Fifty rads stimulated BFU-E values to 250 % of control fetuses. However, 100 rads was sufficient to reduce BFU-E to almost 10 % of 0-rad values.

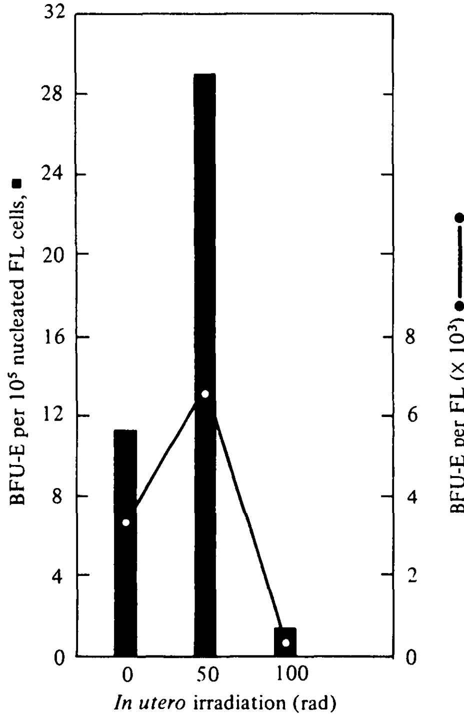

Fig. 3. Effect of low-dose irradiation on BFU-E per 10^5 fetal liver cells and per fetal liver. HA/ICR fetuses were irradiated *in utero* on day 10·5 of gestation. Plasma clot cultures contained 25000 day-14·5 fetal liver cells per 0·1 ml and 3·0 units of erythropoietin per ml.

Fetal liver granulocyte and macrophage progenitor cells

The nontreated day-14·5 fetal liver had more GM-CFC than any of the irradiated fetuses (Fig. 4). There was a continuous reduction in recovery of GM-CFC per fetal liver with each increase in dose of irradiation. Although the M-CFC concentration (per 10^5 fetal liver cells) remained relatively unchanged with increasing doses, there was a significant increase in M-CFC per organ with 50 rads, followed by a continuous dramatic drop in M-CFC values with each increase in dose (Fig. 5).

DISCUSSION

Exposure of the fetus to low-dose ionizing radiation during active organogenesis was shown to have dramatic effects on the growth of fetal liver tissue and fetal liver hemopoiesis reflected by both a retardation in growth of tissue size and suppressed erythropoietic and granulopoietic progenitor cell activity. Results from our micro plasma-clot cultures for day-14·5 fetal liver are in accordance with values for CFU-E and BFU-E reported by other investigators (Dunn *et al.* 1978; Rich & Kubanek, 1976, 1979), with the exception of a greater occurrence of EPO-independent CFU-E in our cultures. In our laboratory, adult

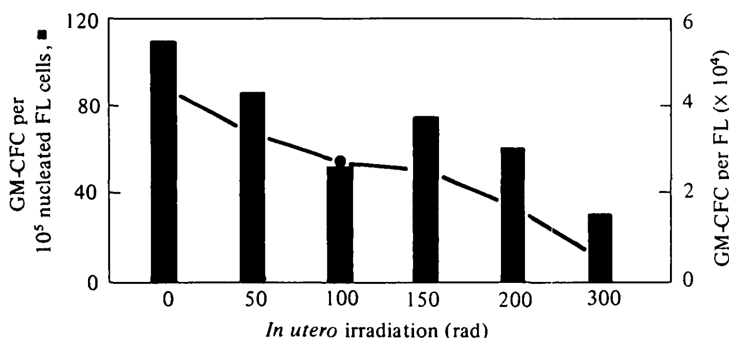

Fig. 4. Effect of low-dose irradiation on GM-CFC per 10^5 day-14·5 fetal liver cells and per fetal liver, HA/ICR fetuses were irradiated *in utero* on day 10·5 of gestation. Double-layer soft agar cultures contained 5×10^4 cells per ml and PMUE as CSA.

bone marrow cultures in the absence of EPO plated in identical conditions results in about 2 CFU-E per 10^5 marrow cells, compared to about 500 CFU-E per 10^5 marrow cells when 0·125 units of EPO per ml is added to the culture medium. Two different batches of fetal bovine serum (from Reheis Chemical Co. and Flow Laboratories, Inc.) were compared to assess the possibility of EPO in the control cultures, which may have accounted for the observed exceptionally high numbers of EPO-independent CFU-E. However, there was no difference in EPO-independent CFU-E values in cultures prepared with either source of serum. Consequently, negligible amounts present in all commercially prepared fetal bovine serum used in culture media may be sufficient for expression of colony formation. The evidence strongly suggests the existence of two populations of EPO-sensitive cells in the day-14·5 fetal liver, one of which is probably a transient population during the hepatic stage of hemopoietic embryogenesis. Earlier reports (Chui, Djaldetti, Marks & Rifkind, 1971; Rifkind, Chui, Djaldetti & Marks, 1969; Tarbutt & Cole, 1970) of hemoglobin synthesis, cell population, and ^{59}Fe-uptake following addition of EPO to fetal liver cells *in vitro* were interpreted to mean that the hepatic hematopoietic stage comprised two waves of erythropoiesis: an EPO-sensitive phase, followed by a transient EPO-insensitive phase commencing at about day 15 of gestation. More recent investigations by Zanjani, Poster, Mann & Wasserman (1977) with fetal sheep and fetal goat (using the latest, more sensitive tissue culture assays to monitor erythroid response to EPO) demonstrated the existence of EPO-dependent and EPO-independent subpopulations of erythroid progenitor cells (CFU-E). It has been shown (Zanjani *et al.* 1977) that sheep fetal hemopoietic tissues (i.e. liver, spleen, and bone marrow) contain cells capable of erythroid activity *in vitro* in the absence of EPO. However, these EPO-independent cells were not present in adult sheep bone marrow cells cultured under the same conditions. It has been hypothesized that these EPO-independent CFU-E are instead '*in vivo* endogenous CFU-E' exhibiting a greater sensitivity to EPO because erythropoietin

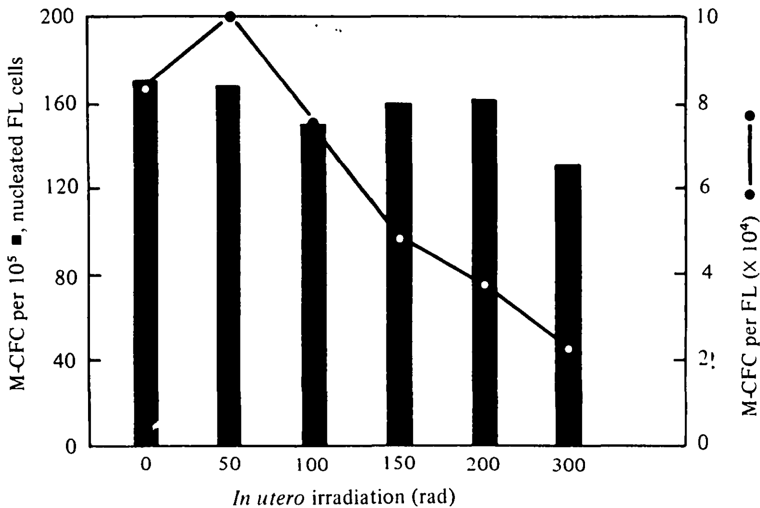

Fig. 5. Effect of low-dose irradiation on M-CFC per 10^5 day-14·5 fetal liver cells and per fetal liver. HA/ICR fetuses were irradiated *in utero* on day 10·5 of gestation. Double-layer soft agar cultures contained 5×10^4 cells per ml, and PMUE was added on day 3 of cultures as CSA.

levels in fetal animals are slightly greater than in adult animals (Roodman & Zanjani, 1979).

A similar situation has been recognized with the murine fetal granulocyte-macrophage progenitor cell. Day-14·5 fetal liver appears to have more macrophage colonies in the soft agar cultures (i.e. 171 M-CFC per 10^5 cells following 25 days of culture) than granulocyte-macrophage colonies (i.e. 109 GM-CFC per 10^5 cells following 8 days of culture). Johnson & Metcalf (1978*a*, *b*) compared the physical properties of murine fetal liver GM-CFC to adult marrow GM-CFC. Fetal progenitor cells had a lighter buoyant density, were larger in size, and produced more macrophage colonies. Studies in our laboratory showed that the adult mouse bone marrow have twice as many GM-CFC as the fetal liver (i.e. adult virgin female bone marrow = 209 GM-CFC per 10^5 and pregnant mouse bone marrow = 234 GM-CFC per 10^5). This is in contrast to the observations of Johnson & Metcalf (1978*b*), who reported fetal liver to be twice as active as adult bone marrow, and to the data of Rickard, Shadduck, Howard & Stohlman (1970), who reported the number of CFU-C per 10^5 of bone marrow and fetal liver to be similar, with the bone narrow slightly more active. The differences recorded by each laboratory are most likely due to the different CSA used in the agar cultures and to the different strains of mice investigated.

Experimentally induced perturbations of hematopoiesis in the fetus have received the attention of other investigators. Jacobson, Marks & Gatson (1959) monitored the changes in ^{59}Fe-uptake, morphology of blood cells, and histology of blood-forming tissues in the fetuses at birth of polycythemic mothers.

Zanjani conducted investigations with fetal sheep demonstrating increased percent ^{59}Fe-uptake, reticulocyte levels, and hematocrit levels as well as erythropoietin production in anephric sheep fetuses following testosterone or thyroxin administration (Zanjani & Banisadre, 1979). Two forms of anemic hypoxia in fetal sheep (i.e. hemolytic anemia induced by phenylhydrazine hydrochloride and anemia caused by phlebotomy) were potent stimuli to increase erythropoietin production and all hematological parameters in the fetus (Zanjani *et al.* 1977). Kubanek, Bock, Bock & Heit (1975) used a high dose of an S-phase-specific cytostatic agent, hydroxyurea, to ascertain stem cell recovery in the day-13 fetal mouse. Erythropoiesis in the fetal liver was shown to be more heavily damaged than was myelopoiesis, and the pluripotential stem cell (CFU-S) had the ability to recover more rapidly, compared to the adult response. Self-replication of CFU-S occurred before differentiation, a response contrasting to that of adult bone marrow. Tissue cellularity and *in vitro* response to EPO also increased after hydroxyurea treatment.

Our studies with ionizing radiation showed fetal liver granulopoiesis to be more radiosensitive to injury compared to erythropoiesis and to have a greater potential for erythropoiesis recovery. This should not be unexpected inasmuch as the day-14·5 fetal liver is greater than 80% erythropoietically active, as indicated by the cytosmear preparations of the fetal liver cell suspensions and the high numbers of CFU-E per 10^5 fetal liver cells compared to adult bone marrow and spleen (i.e. 1572 CFU-E per 10^5 fetal liver cells, 531 CFU-E per 10^5 adult bone marrow cells, and 71 CFU-E per 10^5 spleen cells).

It is clearly evident that study of the embryogenesis of hemopoiesis has gained renewed interest since the time of the early hematology morphologists, due to development of new technology to assess cellular activity and migration of cells during the ontogeny of the different hemopoietic loci. Further studies are being conducted in our laboratory to evaluate latent effects of *in utero* low-dose irradiation during the early stages of gestation on the hemopoiesis of the neonate.

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REFERENCES

Barker, J. E. (1970). Embryonic mouse peripheral blood colony-forming units. *Nature* **228**, 1305–1306.

Brent, R. L. (1971). The response of the 9-1/2-day-old-rat embryos to variations in exposure rate of 150R X-irradiation. *Radiat. Res.* **45**, 127–136.

Chui, D. H. K., Djaldetti, M., Marks, P. A. & Rifkind, R. A. (1971). Erythropoietin effects on fetal mouse erythroid cells. I. Cell population and hemoglobin synthesis. *J. Cell Biol.* **51**, 585–595.

Djaldetti, M., Goldman, J. A. & Fishman, P. (1978). Ultrastructure of the cells in the peripheral blood of human embryos at early gestational stages. *Biol. Neonate* **33**, 177–183.

DUNN, C. D. R., NAPIER, J. A. F., FORD, T. W., PRICE, V. A. & MITCHELL, J. M. (1978). Separation of erythropoietin responsive cells in fetal mouse liver. *Acta Haematol.* **59**, 223–230.

JACOBSON, L. O., MARKS, E. K. & GATSON, E. O. (1959). Studies on erythropoiesis. XII. The effect of transfusion-induced polycythemia in the mother on the fetus. *Blood* **14**, 644–653.

JOHNSON, G. R. & METCALF, D. (1978*a*). Clonal analysis *in vitro* of fetal hepatic hematopoiesis. In *Differentiation of Normal and Neoplastic Hematopoietic Cells*, vol. 5 (ed. B. Clarkson, P. A. Marks & J. E. Till), pp. 49–62. Cold Spring Harbor Conferences on Cell Proliferation.

JOHNSON, G. R. & METCALF, D. (1978*b*). Sources and nature of granulocyte-macrophage colony-stimulating factor in fetal mice. *Expl Hematol.* **6**, 327–335.

JOHNSON, G. R. & MOORE, M. A. S. (1975). Role of stem cell migration in initiation of mouse foetal liver haemopoiesis. *Nature* **258**, 726–728.

KUBANEK, B., BOCK, E., BOCK, O. & HEIT, W. (1975). Growth patterns of foetal hemopoiesis after injury. In *Erythropoiesis* (ed. K. Nakao, J. W. Fisher & F. Takaku), pp. 371–379. Baltimore: University Park Press.

MACVITTIE, T. J. (1979). Alterations induced in macrophages and granulocyte-macrophage colony-forming cells by a single injection of mice with *Corynebacterium parvum*. *J. Reticuloendothel. Soc.* **25**, 479–490.

MARTIN, P. G. (1973). Postnatal growth in prenatally irradiated rats. I. Exposure rate effect on the liver, kidney, spleen and thymus. *Growth* **37**, 55–67.

MCLEOD, D. L., SHREEVE, M. M. & AXELRAD, A. A. (1974). Improved plasma clot culture system for production of erythrocytic colonies *in vitro:* Quantitative assay method for CFU-E. *Blood* **44**, 517–543.

METCALF, D. & MOORE, M. A. S. (1971). *Haemopoietic Cells*, pp. 172–271. New York: American Elsevier.

POZZI, L. V., ANDREOZZI, U. & SILINI, G. (1972). Colony forming units in fetal spleen and peripheral blood. Data for a comparative elevation of pre- and post-natal haemopoiesis. *Acta Haematol.* **48**, 337–346.

RICH, I. N. & KUBANEK, B. (1976). Erythroid colony formation (CFU-E) in fetal liver and adult bone marrow and spleen from the mouse. *Blut* **33**, 171–180.

RICH, I. N. & KUBANEK, B. (1979). The ontogeny of erythropoiesis in the mouse detected by the erythroid-colony-forming technique. I. Hepatic and maternal erythropoiesis. *J. Embryol. exp. Morph.* **50**, 57–74.

RICKARD, K. A., SHADDUCK, R. K., HOWARD, D. E. & STOHLMAN, Jr., F. (1970). The granulocytic *in vitro* growth potential of foetal liver. *Pathology* **2**, 297–306.

RIFKIND, R. A., CHUI, D., DJALDETTI, M. & MARKS, P. A. (1969). Fetal erythropoiesis: Erythropoietin effects on cultured cells. *Trans. Assoc. Am. Physicians* **82**, 380–387.

RUGH, R. (1971). X-ray induced teratogenesis in the mouse and its possible significance to man. *Radiology* **99**, 433–443.

ROODMAN, G. D. & ZANJANI, E. D. (1979). Endogenous erythroid-colony-forming cells in fetal and newborn sheep. *J. Lab. Clin. Med.* **94**, 699–707.

TARBUTT, R. G. & COLE, R. J. (1970). Cell population kinetics of etythroid tissue in the liver of foetal mice. *J. Embryol. exp. Morph.* **24**, 429–446.

TUBIANA, M. (1979). Problèmes posés par l'irradiation des femmes enceintes effets des radiations ionisantes sur l'embryon et le foetus. *Bull. Cancer (Paris)* **66**, 155–164.

WEINBERG, S. R. & STOHLMAN, Jr., F. (1976). Growth of mouse yolk sac cells cultured *in vitro*. *Br. J. Haematol.* **32**, 543–555.

YOFFEY, J. M. (1971). The stem cell problem in the fetus. *Isr. J. Med. Sci.* **7**, 825–833.

ZANJANI, E. D. & BANISADRE, M. (1979). Hormonal stimulation of erythropoietin production and erythropoiesis in anephric sheep fetuses. *J. Clin. Invest.* **64**, 1181–1187.

ZANJANI, E. D., POSTER, J., MANN, L. I. & WASSERMAN, L. R. (1977). Regulation of erythropoiesis in the fetus. In *Kidney Hormones* (ed. J. W. Fisher), pp. 463–493. New York: Academic Press.

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