Fetal Response to Injury in the Rabbit

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● **Fetal, neonatal, and adult tissue response to a standardized injury was studied using subcutaneous wound implants, linear incisions, and punch wounds in New Zealand white rabbits. In the fetus, sutured incisions healed by primary intention without antecedent inflammation. However, neither contraction nor healing by secondary intention was seen in punch or unsutured wounds. Healing both by primary and secondary intention following inflammatory infiltration was observed uniformly in neonatal and adult rabbits. Wound implants were extensively infiltrated with collagen in the adults studied; however, no collagen was seen in fetal implants and collagen hydroxyproline content could not even be detected by high performance liquid chromatography techniques; rather, a matrix rich in hyaluronic acid was found. The fetal tissue response to injury differs from the adult, proceeding in the absence of a classical inflammatory stimulus and lacking contractile capabilities. The deposition of extracellular matrix rich in hyaluronic acid but devoid of collagen suggests that the fetal response to injury may be a process more closely resembling regeneration or growth rather than repair by scar deposition.**

INDEX WORDS: Wound healing.

EXISTING CLINICAL DATA suggest that the very young respond to injury in a far different fashion than the adult. For example, there has been a long-standing empiric observation that the neonate seldom demonstrates wound healing problems. Extending clinical observations to the fetus, Rowlatt[1] was able to examine wounds in a 20-week human abortus; she noted that inflammatory cells and fibroblasts, the usual cellular response in the adult, were absent. Instead, healing was accomplished by mesenchymal proliferation.

Investigative work thus far corroborates these observations. Burrington,[2] Rowsell,[3] Hallock,[4] and Adzick et al[5] all demonstrated distinct, though conflicting differences between the fetal and adult tissue response to injury in several animal species. All noted the lack of a classic inflammatory response following injury; observations of the reparative events were less consistent.

Thus, it might be hypothesized that, the cellular and connective tissue responses to injury in the fetus are distinctly different from those of the adult.

To directly test this hypothesis, the tissue response to injury was studied in fetal, neonatal, and adult New Zealand white rabbits using several standard wound models.

MATERIALS AND METHODS

Time-dated pregnant New Zealand white rabbits were obtained from Hazelton Research Products (Denver, PA) and housed locally for 3 to 5 days prior to study. Fetal wounding studies were performed on the 22nd to 24th day of gestation (term 31 days). Several pregnant does delivered litters at term; surviving newborns were then wounded on the second day of life. Nine-month-old nonpregnant rabbits weighing over 10 lb were used to provide a mature adult response for comparison. One of three standardized wounds were placed in each animal studied.

Wounds

(1) Two paravertebral linear 1 cm incisions were made with a no. 11 knife blade; one incision was sutured, the other was left open.

(2) A 4-mm full-thickness punch wound was created with a biopsy punch.

(3) Subcutaneous wound implants as described by Diegelmann et al[6] consisting of a 12 × 2 mm perforated silicone tube (Wound Healing Tube, Mentor-Heyer Schulte, Goleta, CA, No. 82-0034-01) with and without polyvinyl alcohol (PVA) sponge inserts[7] (cut from Merocel Surgical Sponges, Mentor, Hingham, MA, No. 22-3620) were placed paravertebrally.

Wounding Techniques

Fetus. Fetal surgical techniques perfected and described by Adzick and Harrison[8] were replicated. Important considerations included general anesthesia with spontaneous halothane/oxygen ventilation by nose cone, midline maternal laparotomy, antimesenteric hysterotomy within a full-thickness purse-string suture and gentle manipulation of the fetal hindquarters into the hysterotomy for wounding. The hysterotomy was then closed by tying the purse-string suture following reconstitution of the amniotic fluid volume with warmed plasmalyte solution (Travenol Laboratories Inc, Deerfield, IL; Figs 1 and 2). Fetal survival following wounding was 84% in 106 fetuses.

Neonate. Two-day-old rabbits had wounds created or implants placed paravertebrally using 0.5% lidocaine local anesthesia. Neonatal survival was 95%.

Adult. Nonpregnant adult rabbits were wounded or implanted in identical fashion using the general anesthetic technique employed for the pregnant rabbits. There were no deaths.

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Wound Harvest

Wounds/implants were excised 1 to 17 days postoperatively in the neonate and adult under general anesthesia. The animals were then killed with pentobarbital. Fetuses were delivered by cesarean section 1 to 7 days after wounding and subsequently killed by halothane inhalation.

All specimens were harvested; care was taken to ascertain fetal viability prior to their inclusion. Specimens were fixed immediately in 10% buffered formalin, processed and examined with standard histologic methods. A total of 89 fetal wounds, 38 neonatal wounds, and 11 adult wounds were available for study.

PVA implant samples for quantitation of hydroxyproline were frozen at −70°C until the time of assay by the derivitization and high performance liquid chromatography (HPLC) technique of Lindblad and Diegelmann.[9] Following hydrolysis in 6N HCl (120°C, 15 psi, 14 hours), the protein hydrolysate was evaporated to dryness and then redissolved in a minimal volume of deionized water for analysis (sensitivity 40 pmol).

Implants used for quantitation of glycosaminoglycans were stored at −70°C, then digested using proteinase K and extracted as described by Smith et al.[10] Total glycosaminoglycan was determined by Alcian blue staining and measurement of the optical density at a wavelength of 620 nm.[10] Individual glycosaminoglycans were separated using cellulose acetate electrophoresis.

RESULTS

Surgical Wounds

In the fetus, sutured incisions consistently demonstrated healing by primary intention at seven days post wounding. Early sections did not show polymorphonuclear leukocyte (PMN) infiltration. By day 7, though gross observation suggested complete healing, a clot persisted in the wound with viable mononuclear cells enmeshed within the clot and epithelialization was not completed. Ingrowth of mesenchymal cells from the lateral margins of the wound was apparent; however, very few fibroblasts were seen and only minimal collagen was seen on the Masson's trichrome stained sections.

Unsutured wounds or punch wounds gaped open in the fetus and actually increased in size. There was no apparent contraction or healing by secondary intention. Cellular events were otherwise similar to those seen in sutured wounds; PMNs and fibroblasts were absent.

In neonatal and adult rabbits, healing by primary and secondary intention was uniformly noted. Acute inflammatory cells predominated early, followed by migration of fibroblasts with subsequent deposition of collagen.

Wound Implants

Hollow silicone tube implants from the fetus contained small round inflammatory cells, with progressive accumulation of matrix material at seven days. This matrix material did not appear to be fibrin reticulin or collagen as determined by specific staining.

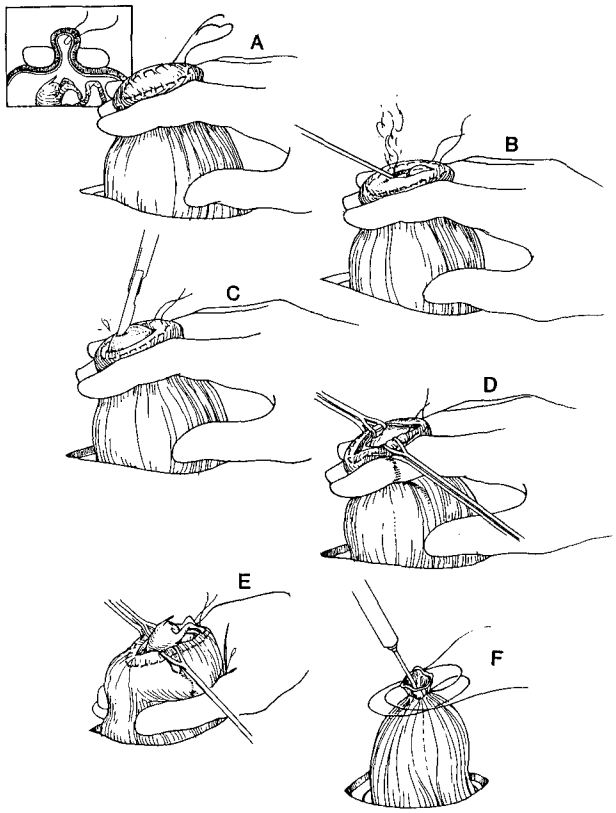

Fig 1. Technique of hysterotomy. (A) Purse-string suture incorporating all layers. (B) Myometrium is opened with the cautery; a bleb of amnion rises. (C) Amnion is opened. (D) Babcock clamps control uterine wall and fetal membranes. (E) Fetal hindquarters exposed or wounding. (F) Fetus returned to the uterus; amniotic fluid volume reconstituted.

Instead, there appeared to be positive staining for mucosubstances with Alcian blue staining; pretreatment of these sections with the enzyme hyaluronidase eliminated the staining with Alcian blue, thus suggesting the presence of hyaluronic acid.

There was no accumulation of any cellular or matrix material in adults compared with hollow silicone tube. Neonatal implants showed scant matrix.

PVA implants provoked an early acute inflammatory response and were extensively infiltrated with fibroblasts and collagen by 7 to 10 days in the adults studied (Figs 3A and 4A). In contrast, no acute inflammatory cells were seen in the fetal PVA implants, rather small round mononuclear cells predominated both early and late. Few fibroblasts were seen (Fig 3B), and there was no apparent collagen accumulation on trichrome stained sections (Fig 4B).

PVA implants harvested from fetuses at seven days and assayed for collagen hydroxyproline by the HPLC technique showed no detectable collagen.

Fetal PVA specimens analyzed for glycosaminoglycans demonstrated four times the accumulation of glycosaminoglycans compared with adult rabbits stud-

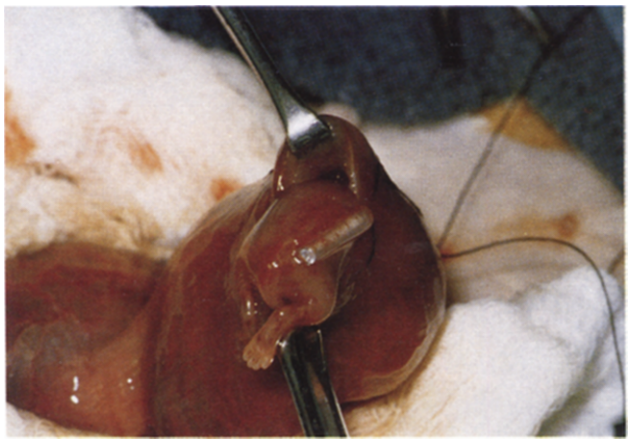

Fig 2. Fetal rabbit (22 days) with subcutaneous PVA implant.

ied. This quantity is ten times the amount of glycosaminoglycans found in fetal skin. Preliminary isolation of individual glycosaminoglycans by cellulose acetate electrophoresis confirmed that most of the glycosaminoglycans is indeed hyaluronic acid.

DISCUSSION

Despite obvious similarities, there are fundamental intrinsic and extrinsic (environmental) differences between the fetus and the adult that certainly impact on wound healing. The protective environment inhabited by the fetus is not only sterile, the skin is continuously bathed in amniotic fluid that may contain peptide growth factors.[11]

Intrinsic differences are even more remarkable as may be related to wound healing. The fetus itself is preprogramed for extraordinary and continuous growth, unparalled at any other time in its existence. Immunologically, the fetus is much less competent than the adult; not only is there neutropenia[5,12] but cellular immunity is virginal in its antigenic experience, and there may be a defect in chemotactic ability,[13] though phagocytic function is probably normal.[14] Finally, the fetus is notably hypoxic with arterial pO_2 $\leq$20 torr.[15] All of these factors must affect the cellular and connective tissue response to injury.

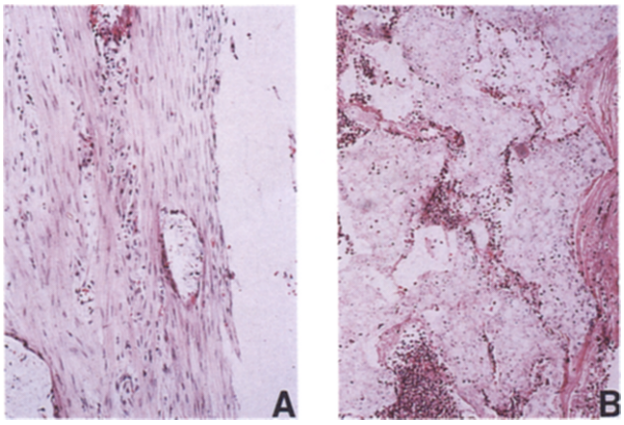

Fig 3. (A) PVA implant from adult rabbit at seven days (H&E, ×48). Note extensive fibroblast infiltration. (B) PVA implant from fetal rabbit at seven days (H&E, ×48). No fibroblasts are present.

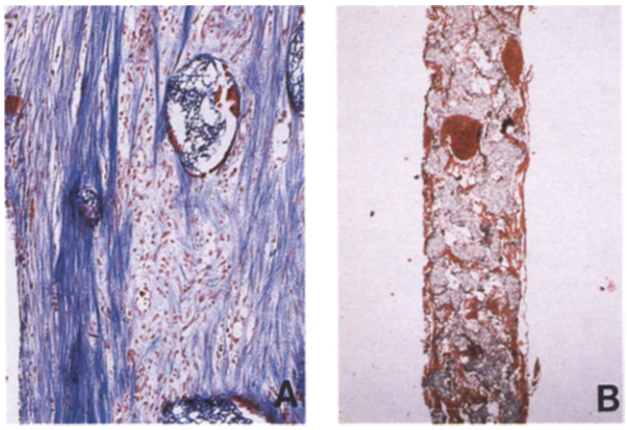

Fig 4. (A) PVA implant from adult rabbit at seven days (Masson's trichrome, ×48). Abundant blue collagen seen. (B) PVA implant from fetal rabbit at seven days (Masson's trichrome, ×16). No collagen stained, noncollagen matrix stains red. (PVA sponge material itself does appear gray.)

The study of healing using incisional wounds has a number of inherent limitations, none the less, it is perhaps the least artificial of any wounding technique. Incisional wounding in the fetus demonstrated fundamental differences in both the cellular and connective tissue events when compared with the adult events of healing. Following incisional injury, acute infiltration of the wound site with PMNs does not occur in the fetus. Instead, cells that appear to be chronic inflammatory cells arrive, followed by slow ingrowth of mesenchymal elements; epithelialization is slow. Fibroblasts, critical to adult healing, do not partici-

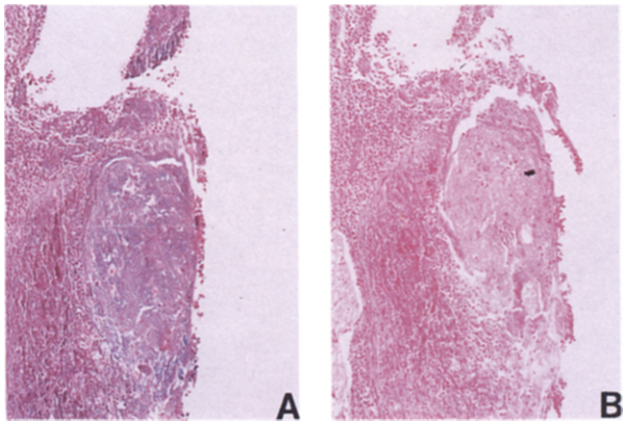

Fig 5. (A) PVA implant from fetal rabbit at seven days (Alcian blue, ×48) blue staining matrix is positive for mucosubstances. (B) Same slide pretreated with hyaluronidase, then stained (Alcian blue, ×48). Matrix no longer stains positive, suggesting that matrix is hyaluronic acid, a specific glycosaminoglycan.

pate, and collagen deposition is not seen in fetal incisional healing in sutured wounds. These findings are comparable to the histologic evaluations of skin incisions by Hess,[16] Burrington,[2] Goss,[17] Robinson and Goss,[18] Rowsell,[3] Hallock,[4] and Adzick et al.[5] Fetal incisions do not heal by scar deposition.

In this rabbit model, neither unsutured wounds nor punch wounds showed evidence of contraction, and in fact, they gaped open. Somasundaram[19] has made the identical observation, whereas Burrington[2] noted rapid contraction of fetal sheep wounds at 90 days gestation (similar early third trimester). Such discrepancy may be species related; however, this consistent observation in the rabbit warrants further investigation.

Although gross and microscopic examination of surgical wounds provides some useful information, analysis is limited by the inability to quantitate any parameter. Thus, a number of wound implant devices have been constructed including the Hunt-Schilling chamber,[20,21] the Celstice device,[22] Goodson and Hunt's expanded polytetrafluoroethylene tube,[23] and the PVA implant.[6] For fetal studies in the rabbit, only the Gortex tube or the PVA implant meet the technical and spatial demands.

Encapsulation and poor penetration of the Gortex tube model and extensive experience with the PVA implant led to its use in this experiment. Histologic events in the PVA implant were comparable to those seen in surgical incisions. No collagen was seen histologically and assay for hydroxyproline by HPLC techniques failed to demonstrate even minute quantities of collagen. Interestingly, in a similar model using the Gortex implant, Adzick et al[5] noted substantial accumulation of hydroxyproline in fetal implants compared with neonates and adults studied. This discrepancy may be related to the type of implant used.

The histologic and quantitative demonstration of hyaluronic acid, one of the glycosaminoglycans (ground substance) is of some interest. Glycosaminoglycans are one of the three major extracellular matrix marcomolecules along with fibrin and collagen. The glycosaminoglycan role in adult healing is transitory,[24] providing a provisional matrix following fibrin degradation in a healing wound only to be subsequently replaced by collagen. The observations in this model suggest that previous observations of fetal healing without scar formation[1-4,16-19] may be accounted for by a prominent role for mesenchymal ingrowth within a glycosaminoglycan matrix in fetal wounds. Thus, the fetal cellular and connective response to injury may be a process more closely resembling regeneration and growth rather than repair by collagen deposition. As such, these events may serve to bridge the gap between regenerative phenomena in the embryo and classic mammalian healing by scar deposition in the adult.

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