# REACTION OF RAT EMBRYONIC TISSUES TO MECHANICAL INJURY AND INFECTION WITH Staphlococcus Aureus (strain 209)

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Healing of skin wounds and the tissue reaction to intrauterine injection of Staphylococcus aureus (strain 209) were studied in experiments on albino rat embryos. With a horizontal incision, an orthotonic position of the fetus and retraction of the skeletal muscles prevented contraction of the wound and epithelization of its surface. With a vertical incision the wound edges approximated and healing took place without the formation of granulation tissue by epithelization of the wound surface 2 days after the operation. The embryo responded to injection of the pathogenic staphylococcus by a uniform local reaction with predominance of degenerative changes and accumulation of histiocyte-like cells close to some of the colonies. No exudative inflammatory reaction was present either to trauma or to injection of the staphylococcus at all stages of intrauterine life.

KEY WORDS: fetus; wound healing; infection with Staphylococcus aureus (strain 209).

The healing of skin wounds has been closely investigated, but only in the postnatal period. Information in the literature on the tissue response of mammalian fetuses to trauma is contradictory. Some workers [2, 4, 5] have observed the appearance of granulation tissue after injury to guinea pig and rabbit fetuses. This is contrary to observations by other workers [6] who did not observe granulation tissue formation or epithelization of the wound surface in experiments on rabbit embryos.

Bacteriemia has been observed after injection of a culture of staphylococci into the amniotic fluid, placenta, and embryonic tissues. The fetus remains morphologically anergic for a long time against bacterial irritants. The cellular defensive reaction of the embryo was absent in the early stages of development and very weak in the later period of intrauterine life [3, 7, 8].

The object of this investigation was to study healing of skin wounds of rat fetuses and to investigate the response of their tissues to intrauterine injection of Staphylococcus aureus (strain 209). Advances in the surgery of the premature infant have made theoretical aspects of repair in fetuses a matter of practical importance.

## EXPERIMENTAL METHOD

Operations on pregnant noninbred albino rats were carried out under general ether–oxygen anesthesia. The bicornuate uterus of the rats was exposed through a midline laparotomy incision and withdrawn from the peritoneal cavity. Experiments were carried out on 228 10-20-day embryos (pregnancy in the rat lasts 21-22 days). In operations on 137 embryos the uterus was incised and the membranes divided with an ophthalmic scalpel above the dorsum of the fetus, which could be seen through the wall of the uterine cornu. With the same scalpel an incision 0.2-0.4-cm long was made in the skin of the dorsal part of the fetus, brought into the wound in the uterine wall. In 97 cases the operation wounds were made perpendicularly to

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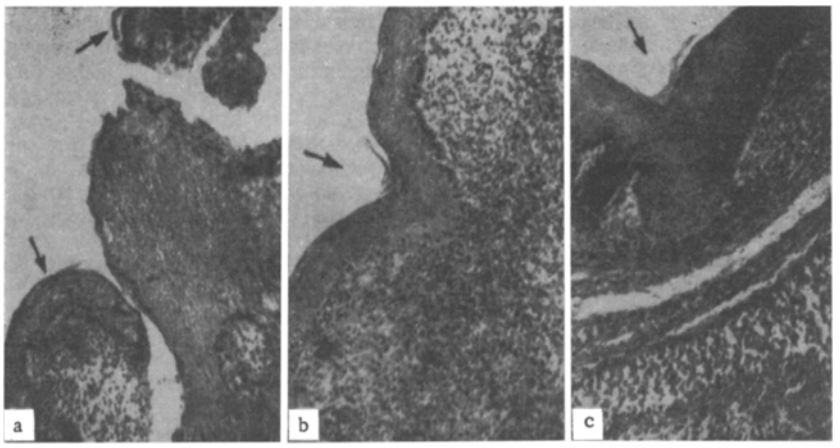


Fig. 1. Healing of skin wounds during antenatal ontogeny of rats (72 ×): a) 19-day rat fetus 1 day after operation: wound edges (shown by arrows) approximated after vertical incision; blood clot below; proliferation of epithelium absent; b) 17-day fetus, 2 days after operation: healed wound covered with stratified squamous epithelium, penetrating into underlying connective tissue (arrow), in which there is a focus of round-cell infiltration; c) epithelized wound of 18-day fetus on 3rd day after operation: stratified squamous epithelium much thicker and penetrates deeply into subepithelial connective tissue.

the long axis of the embryo (horizontally), and in 40 cases parallel to it. The wounds of the embryos were left open; bleeding from these wounds was very slight. A suspension of the microorganisms was injected into the tissues of 91 embryos by puncture of the uterus and of the membranes above the dorsal region of the fetus, and a suspension of a 24-h culture of Staphylococcus aureus (strain 209), containing $1.25 \cdot 10^9$ bacterial cells per ml, was injected subcutaneously into the lower third of the dorsum of the embryo in a dose of 0.025 ml/g body weight. To determine the exact dose of staphylococcal culture injected, one of the embryos from each mother rat was removed during the operation from the amniotic sac and weighed. The uterus was replaced in the peritoneal cavity and the wound of the abdominal wall sutured in layers without drainage. The mother rats recovered after the operation and no complications were observed. In most cases pregnancy continued. Fetuses were taken from the uterus by caesarian section for morphological study of the wounds 1, 2, 3, and 4 days after mechanical injury and 0.5, 1, 2, and 3 days after injection of the staphylococci. The material was fixed in 10% neutral formalin. Paraffin sections 7–8 $\mu$ thick were stained with hematoxylin–eosin, by the methods of Van Gieson and Gram–Weigert, and impregnated with silver by Foot's method; fibrin was demonstrated by Shueninov's method, and elastic fibers were stained by Hart's method.

## EXPERIMENTAL RESULTS

Of the 228 experimental fetuses 160 (70%) were alive at the time of investigation. Death of 68 fetuses (30%) took place most frequently on the 10th–13th day of intrauterine life. A second examination of the uterus showed that in 19 cases prenatal death of the fetuses had occurred with maceration, but with preservation of their outlines. In the remaining 49 cases the outlines were not preserved and the fetuses had undergone partial resorption and were converted into an amorphous, structureless mass which, on histological examination, consisted of a chaotic arrangement of cells with nuclei in a state of karyopycnosis and karyorrhexis.

Healing of the skin wounds inflicted on the albino rat fetuses depended on the position of the operation incision on the dorsum of the fetus. If the wounds were horizontal in direction, defects of the skin cover were observed on macroscopic examination and were of the same size as the operation incisions. The wound edges of all fetuses, irrespective of their age and the duration of the experiment, appeared smooth. The wounds "gaped," i.e., no contraction was observed. The wound surface was covered with elongated spindle-shaped cells with oval and round nuclei. The epithelium terminated abruptly at the wound edges and did not "creep" over the surface of the incision, so that the injuries themselves looked like punched-out defects in the tissue. In some cases a local connective-tissue macrophagal response was observed at the

wound edges. The blood vessels in the region of injury appeared dilated and filled with erythrocytes; polymorphs were completely absent in the lumen of the blood vessels, on the wound surfaces, and in the underlying connective tissue. In the case of a vertical incision, 24 h after the operation marked contraction of the wounds was observed: only a small slit-like defect remained at the site of the operation incision. Very slight nonapposition of the epithelium was present; a small blood clot was present between the approximated and thickened wound edges. The floor of the wound was covered by groups of oblong connective-tissue cells. No leukocytic infiltration or formation of new blood vessels was observed (Fig. 1a). After 2 days the wound appeared healed: at the site of injury a gutter-like hollow was observed and on histological examination this was seen to be covered with thickened stratified squamous epithelium, penetrating into the underlying tissue. Beneath the epidermis lay a moderately large cluster of fibroblasts together with single round cells of lymphocyte type (Fig. 1b). On the 3rd day the depression at the site of the operation incision was much shallower than on the 2nd day; it was difficult to distinguish under the magnifying glass and was almost indistinguishable from the surrounding skin. The epithelium covering the wound completely was highly hyperplastic and penetrated deeply into the underlying subepithelial connective tissue (Fig. 1c).

Morphological study of fetuses receiving a subcutaneous injection of a culture of Staphylococcus aureus (strain 209) showed numerous colonies of staphylococci beneath the skin, in the muscles, and in the connective tissue at the site of injection. Degenerative changes were observed in cells around the aggregations of microorganisms and in the immediate vicinity of the colonies: granulation of the cytoplasm, karyorrhexis, and karyopycnosis. At the periphery of some of the necrotic foci a local macrophagal response of the surrounding connective tissue was observed in the form of an accumulation of round and elongated histiocyte-like cells. The connective-tissue fibers in the zone of localization of the bacteria were necrotic and a little edematous. The blood vessels of the dermis were dilated not only at the site of injection of the staphylococci, but also in more distant parts. No exudative inflammatory reaction was observed.

The results of these experiments thus show that intrauterine healing of skin wounds depends on the direction of the operation incision on the dorsum of the fetus relative to its long axis. With a horizontal operation incision the orthotonic position of the fetus and retraction of the skeletal muscles prevent contraction of the wound and epithelization of its surface. If the operation incision is parallel to the axis of the fetus, the wound edges approximate. Healing takes place without the formation of granulation tissue through direct epithelization of the wound surface 2 days after the operation by regeneration of stratified squamous epithelium. The fetus responds to the pathogenic strain of Staphylococcus aureus by a uniform local reaction with predominance of degenerative changes and the accumulation of histiocyte-like cells at the periphery in the vicinity of some colonies. Arshavskii [1] showed that the inflammatory response could not develop during the antenatal period. In the present morphological investigations no exudative inflammatory response took place either to mechanical trauma or to injection of a culture of Staphylococcus aureus at all periods of intrauterine life.

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