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## Lack of Thymic Effect on Wound Healing.* (30099)

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Much evidence has been presented that the thymus plays an important role in antibody formation, transplantation immunity and delayed hypersensitivity in a variety of species of mammals. This influence has been related to the primacy of fetal and neonatal thymic function for lymphoid development and consequent immunologic competence. Little information is available concerning thymic and lymphocytic function in situations of questionable or non-immunologic nature, such as the inflammatory response. Inflammation following subcutaneous injection of turpentine was observed to be comparable in neonatally thymectomized and intact rats(1). However, mice thymectomized at birth were protected against the lethality of lymphocytic choriomeningitis virus administered one month later. Although virus multiplication was not inhibited, the inflammatory response was suppressed indicating the importance of tissue hyperactivity and the lymphocytic infiltrate in production of symptomatology(2).

This information prompted us to explore the effect of neonatal thymectomy on another model of inflammation and repair, notably wound healing.

*Methods.* Newborn Sprague-Dawley rats were randomized within 12 hours after birth so that one-half of each litter was thymectomized and the remainder utilized as controls. Thymectomy was accomplished through a short longitudinal sternal incision with a wire snare. Clean, but not aseptic precautions were enforced. All animals were placed in individual cages after weaning and maintained with rat chow supplemented with canned dog food, cabbage and apples *ad lib.* A 4 cm surgical incision of the skin of the lower back was made with a scalpel and the wound closed by 4 evenly distributed interrupted silk sutures. A uniform, circular defect was simultaneously produced in the dorsolateral aspect of the thorax with a 6 mm dermal punch in all animals 61 ± 1-72 ± 6 days of age (Table I). Groups were sacrificed at 2, 5, 8, 14 and 21 days following wounding.

Wound contraction was estimated by daily planimetry of the skin defect on the thorax. Tensile strength was determined from uniform transverse strips of areas between the sutures measuring 1 cm in width and 2 cm on each side of the wound. One end was suspended from a ring stand and weights and/or paper cup attached to the other. Water was slowly delivered into the cup until wound disruption occurred. Tensile strength was based on the average of 2 determinations on each wound. (Differences in readings ranged from 12-25 ml water.)

Other portions of the surgical wound and skin defect were fixed in 10% neutral formalin and absolute alcohol, dehydrated and imbedded in paraffin in the usual manner. Sec-

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TABLE I. Body, Spleen and Adrenal Weights and Age at Wounding of Neonatally Thymectomized and Intact Control Rats (Thymectomized/Intact).

| | 2d | 5d | 8d | 14d | 21d |
|---|---|---|---|---|---|
| No. | 6/11 | 6/11 | 7/11 | 9/18 | 7/8 |
| Wt begin (g) | 195/211 | 196/208 | 176/209 | 179/208 | 183/205 |
| " end | 199/211 | 199/211 | 180/222 | 207/229 | 207/241 |
| Age at wounding | 64 ± 6*/63 ± 3 | 66 ± 5/72 ± 6 | 64 ± 8/65 ± 3 | 62 ± 1/64 ± 2 | 61 ± 1/61 |
| Spleen wt (mg) | 501 ± 127/714 ± 150 | 485 ± 87/657 ± 108 | 406 ± 62/632 ± 103 | 416 ± 93/645 ± 100 | 501 ± 92/702 ± 104 |
| Adrenal wt (mg) | 40 ± 9/41 ± 7 | 46 ± 10/49 ± 10 | 44 ± 10/44 ± 4 | 40 ± 8/45 ± 10 | 47 ± 10/41 ± 8 |
| Thymus (mg) | 414 | 305 | 423 | 353 | 383 |

* Standard deviation.

tions were stained with hematoxylin and eosin, Masson trichrome and Wilder reticulum methods for demonstration of reticulum and collagen fibers. These were subjectively graded 1-3+. Amount of acid mucopolysaccharide was similarly graded in sections stained with alcian blue and thionin (1:10,000, pH 4).

The mediastinum was carefully inspected for thymic remnants. Only rats in which thymectomy was complete were considered for analysis. Spleens and adrenals were weighed at time of sacrifice and sections of these organs, as well as ileum, processed as above and stained with hematoxylin and eosin.

*Results.* No statistically significant difference in tensile strength or rate of wound contraction was apparent in thymectomized and intact members (Fig. 1). No qualitative or quantitative difference in reticulum or collagen fibers or dermal acid mucopolysaccharide was observed (Fig. 2). Neutrophils and lymphocytes and mast cells were frequent in wounds of both thymectomized and intact rats at 2 and 5 days, as were histocytes and occasional plasma cells at 5 and 8 days.

Spleen weight was significantly ($P<0.01$) decreased in thymectomized rats and microscopic examination disclosed depletion of lymphocytes of Malpighian bodies as well as Peyer's patches of ileum. No difference in adrenal weight was noted ($P>0.1$).

*Discussion.* The results of this study indicate that the neonatal thymus exerts no influence on the inflammatory, substrate or fibrillar phases of wound healing. The depletion of lymphoid elements in spleen and Peyer's patches of ileum signify that thymectomy was effective in supressing lymphoid development in rats utilized in this study. Further, we have observed tolerance to orthotopic skin homografts from Long-Evans donors in a high percentage of this strain neonatally thymectomized as performed herein. This information indicates that the lymphocytes and lesser numbers of plasma cells observed in the exudative stage (up to 5 days) of wound healing possess little or no immunologic function, at least of the types abolished by thymectomy, in this form of inflamma-

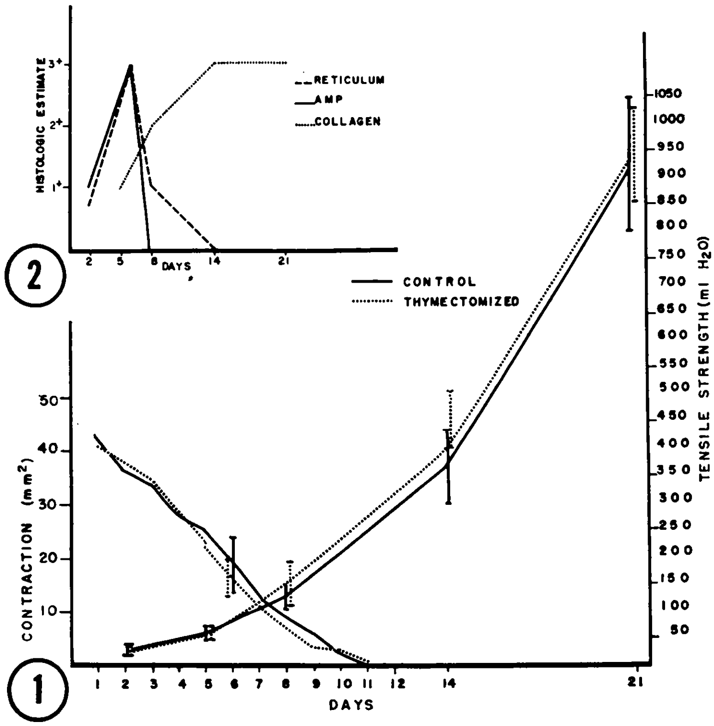


FIG. 1. Tensile strength and rate of contraction of wounds in thymectomized and intact rats.

FIG. 2. Histologic estimation of reticulum, collagen, acid mucopolysaccharide (AMP) of wounds of thymectomized and intact rats. Comparable findings in both groups represented as single lines.

tory response. Normal complement of these cells as well as mast cells in the wounds of thymectomized members also indicates that their presence in this situation is not thymus dependent.

*Summary.* Tensile strength and rate of contraction of standard wounds were comparable in neonatally thymectomized and intact control Sprague-Dawley rats. Quantitative and qualitative histochemical and histological estimation of fibrillar, substrate and exudative stages of wounding were also similar. It is concluded that lymphocytes exert no thymus-dependent immunologic influence on this type of inflammatory response.

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