AFRRI TN73-20
DECEMBER 1973

AFRRI
TECHNICAL
NOTE

A TECHNIQUE FOR THYMECTOMY IN THE ADULT RAT

C. L. Cloud
G. D. Ledney

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Approved for public release; distribution unlimited

AFRRI TN73-20

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

AFRRI TN73-20
December 1973

A TECHNIQUE FOR THYMECTOMY IN THE ADULT RAT

C. L. CLOUD
G. D. LEDNEY

S. J. BAUM
Chairman
Experimental Pathology Department

MYRON I. VARON
Captain MC USN
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Approved for public release; distribution unlimited

ACKNOWLEDGMENT

The authors wish to thank N. A. Eaton for her excellent technical assistance, J. F. Taylor and J. H. Flinton for instruction in surgical technique, and J. M. Cicala for graphic assistance.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

An improved technique for surgical removal of the thymus in the adult rat was developed. Essentially the procedure consisted of forming an aperture to the left of the midline and below the clavicle which minimized excessive trauma and permitted easy aspiration of the thymus. Innovar-Vet was used to produce anesthesia. The animals recovered fully within 2 hours after surgery. Wound clips were removed within 14 days. The survival rate for all rats was 80 percent.

I. INTRODUCTION

Thymectomy of the adult laboratory animal is a useful adjunct to the study of certain immunological processes, such as cell-mediated immunity and graft rejection. Techniques for thymectomizing adult mice,[2,3,8,12] hamsters[11] and rats[1,4,7,9,10,13] have appeared in the literature. However, a detailed description of thymectomy in the adult rat is not available. Thymectomy of the adult rat is difficult to perform due to the close association of the thymus with surrounding connective tissue.

Commonly used anesthetic agents such as Nembutal or ether have been associated with high mortality rates because of difficulties in dosage regulation of Nembutal and impaired respiration due to hypersecretion of mucus following ether anesthesia. Therefore, we sought a fast acting substitute which would assure a quick recovery and obviate undesirable side effects. Innovar-Vet,* an analgesic marketed for dogs and recently used in several other species,[5,6] causes negligible mortality and does not have the side effects of ether or Nembutal. This paper details a technique for thymectomy of adult rats using Innovar-Vet.

II. MATERIALS AND METHODS

Lewis rats (Lew/f Mai), free of chronic murine respiratory virus (CMRV) and weighing 125-150 g, were given subcutaneous injections of 0.04 mg/kg of atropine sulfate (Eli Lilly and Company, Indianapolis, Indiana) 10 min prior to an intramuscular injection of 0.1 ml/kg of Innovar-Vet. This compound is marketed as an analgesic; however, it produces a state of anesthesia which develops within 10 min after injection and lasts about 2 hours.

---

* Innovar-Vet^{R} contains 0.4 mg fentanyl and 20 mg droperidol per ml. Pitman-Moore, Inc., Washington Crossing, New Jersey.

The thoracic and submandibular areas were shaved with animal clippers. A depilatory was not required. The animal was placed in a supine position upon a dissecting board with its head toward the operator. The legs were secured to the operating surface by means of pins and stretched rubber bands placed around the junction of the radio-ulnar and carpal regions and of the tibiofibular and tarsal regions. The neck and chest were moistened with 70 percent ethanol. A longitudinal midline incision was made to the left of the midline through the skin and superficial fascia from the level of the angle of the mandible to the fourth rib (Figure 1).

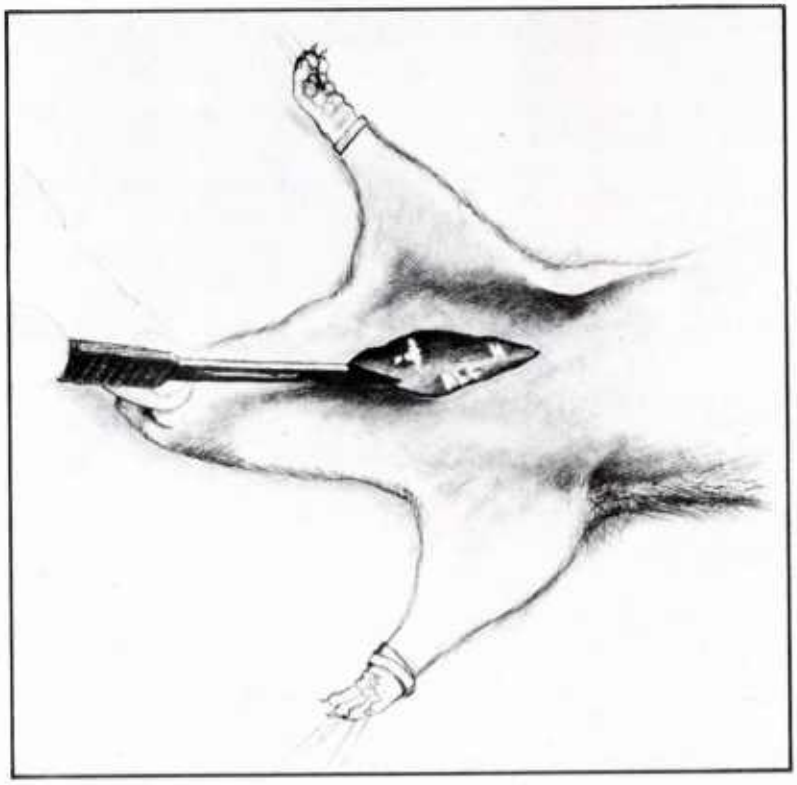

Figure 1. The initial incision is made through the skin and superficial fascia from the level of the angle of the mandible to the fourth rib

Blunt forceps (12 cm) were used to free the skin from the underlying muscle for ease in closure. The pectoralis muscle was transected to the left of the midline starting just below the clavicle and procceding no further than the sccond rib. An incision of this size and at this level facilitated closure and avoided damage to the major blood vessels. The second and third ribs were visualized and clipped and reflected exposing the thymus, which appeared as a glistening organ anterior to the heart. A suction tube was immediately inserted into the chest cavity to aspirate the thymus. Aspiration was assisted by using a toothless iris forceps to divide all areolar tissue connections between the intact gland and the surrounding tissue (Figure 2). Approximately 45 sec were available for aspiration of the thymus. If the thoracic cavity was open for longer than 60 sec, fatal pncumothorax inevitably occurred. If the suction procedure required

Figure 2. Aspiration of the thymus

longer than 45 sec at any particular time, the cavity was closed manually or with 12-cm toothed forceps until the animal resumed normal breathing. A rubber bulb fitted with a two-way valve and a 4-inch piece of Tygon tubing served as a respirator for use in those animals suffering respiratory distress.

Care was taken to avoid injury to the right and left superior vena cava. Some bleeding was expected and was not overly detrimental to recovery. After visual inspection ascertained that no thymic remnants were present, air was expressed from the thoracic cavity by massage directed from the diaphragm toward the head. The thoracic walls were apposed with two 12-cm dressing forceps by the assistant while the operator closed the chest with 4-0 surgical gut medium chromic sutures and a 3/8 circle taper needle. The skin was closed with 9-mm stainless steel wound clips. The animals were then allowed to recover in a cage under a heat lamp at 35°C for 1-2 hours. Within 30 min after closure, the animals regained their normal motor control; the anesthetic effect of the Innovar-Vet subsided completely after 2 hours. Wound clips were removed 14 days after surgery.

The vacuum necessary for aspiration of the thymus was provided by a suction flask attached to a pump or other source capable of providing a pressure equivalent to 16 inches of mercury. Aspiration of the thymus was done with a glass tube which allowed visualization of the organ as it was withdrawn. The glass tube was 16 cm long with an o.d. of 6 mm and an i.d. of 4 mm. A 150° elbow bend was made at the midpoint to facilitate handling, and the ends were fire-polished to avoid trauma. When this procedure was followed precisely as described above, a survival rate of 80 percent was obtained.

REFERENCES

1. Aisenberg, A. C. and Wilkes, B. Immunologic status of thymectomized adult rats. J. Immunol. 93:75-80, 1964.

2. Choudhury, A. and Watkins, E., Jr. Facilitation of complete thymectomy by controlled pneumothorax. Transplantation 7:228-231, 1969.

3. Haimov, M., Wong, D., Burrows, L. and Kark, A. E. Thymectomy in the adult mouse. Transplantation 11:100-102, 1971.

4. Jeejeebhoy, H. F. Immunological studies on the rat thymectomized in adult life. Immunology 9:417-425, 1965.

5. Jones, J. B. and Simmons, M. L. Innovar-Vet^{R} as an intramuscular anesthetic for rats. Lab. Animal Sci. 18:642-643, 1968.

6. Lewis, G. E., Jr. and Jennings, P. B., Jr. Effective sedation of laboratory animals using Innovar-Vet^{R}. Lab. Animal Sci. 22:430-432, 1972.

7. Martin, C. R. Influence of thymectomy on growth of secondary reproductive structures in rats. Am. J. Physiol. 206:193-197, 1964.

8. Ramos, A., Ferreira, A. and Chacón, J. Thymectomy in the adult mouse. Transplantation 12:329-330, 1971.

9. Robson, L. C. and Schwarz, M. R. The influence of adult thymectomy on immunological competence as measured by the mixed lymphocyte reaction. Transplantation 11:465-470, 1971.

10. Segaloff, A. Thymectomy. In: The Rat in Laboratory Investigation, 2nd ed., Farris, E. J. and Griffith, J. Q., Jr., editors, pp. 443-444. New York, N. Y., Hafner Publishing Company, 1949.

11. Sherman, J. D., Adner, M. M. and Dameshek, W. Effect of thymectomy on the golden hamster (Mesocricetus auratus). I. Wasting disease. Blood 22:252-271, 1963.

12. Sjodin, K., Dalmasso, A. P., Smith, J. M. and Martinez, C. Thymectomy in newborn and adult mice. Transplantation 1:521-525, 1963.

13. Vaughan, W. P. and McGregor, D. D. Lymphopoiesis in the rat. II. The effect of thymectomy. J. Cell. Physiol. 80:13-21, 1972.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Armed Forces Radiobiology Research Institute Defense Nuclear Agency Bethesda, Maryland 20014	UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE

A TECHNIQUE FOR THYMECTOMY IN THE ADULT RAT

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

C. L. Cloud and G. D. Ledney

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1973	8	13

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. NWED QAXM	AFRRI TN73-20
c. Task and Subtask C 903	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. Work Unit 03	

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Director Defense Nuclear Agency Washington, D. C. 20305

13. ABSTRACT

An improved technique for surgical removal of the thymus in the adult rat was developed. Essentially the procedure consisted of forming an aperture to the left of the midline and below the clavicle which minimized excessive trauma and permitted easy aspiration of the thymus. Innovar-Vet was used to produce anesthesia. The animals recovered fully within 2 hours after surgery. Wound clips were removed within 14 days. The survival rate for all rats was 80 percent.

DD FORM 1 NOV 65 1473

UNCLASSIFIED
Security Classification