

Thymectomy in Mice : Technical Improvement and Considerations of a Successful Surgical Procedure*

(experimental surgery/mouse thymectomy)

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We have succeeded in eliminating the possibilities of remnants, pneumothorax and hemorrhage during thymectomy by using a head mirror for lighting, and in the adult mice, by detaching the gland with a swab before applying suction. Using superfine silk to close the wound in newborn mice also eliminates cannibalism.

Rapid progress in cellular immunology has revealed diversities in the immune system. T-lymphocytes function as either effector or regulator cells in various immune responses. The role of the thymus in relation to the functionally different peripheral T-cell subsets has been one of the major interests of immunologists. Thus, it has become increasingly important that a reliable surgical technique be devised to remove the organ. Several procedures for thymectomy of mice have already been reported (1—4). These procedures, though generally acceptable, often result in pneumothorax, massive bleeding or incomplete removal of the thymus, as they sometimes involve areas not completely controllable by the operators. The majority of investigators depend on their own experiences, and a reliable and safe technique has long been awaited. We report herein important procedures required for a successful operation and changes we made in the operative procedures which have led to an overall improvement in the technique.

MATERIALS AND METHODS

Thymectomy of Newborn and Young Mice up to 9—10 Days Old

The baby mouse is anesthetized by placing in a refrigerator. The state of hypothermia can be well controlled by watching the exposure time. The mouse is then fastened to a polystyrene board with two pair of rubber bands placed rectangularly. The skin is cleansed with 70% ethanol, and a midline

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longitudinal incision is made from the mid-cervical to the upper thoracic regions. The subcutaneous fascia are divided at the midline and the submaxillary gland is retracted anteriorly, under a binocular dissecting microscope. The exposed sternothyroid and sternomastoid muscles overlying the ventral aspect of the trachea are split open by a pair of swabs. The swab must be thin enough to allow for precise motion but the sharp point of a bamboo pick must be covered by cotton so not to damage vessels in the area. Incision of the middle of the sternum down to the third to fourth rib is made using micro-dissecting scissors. The point of the scissors must be kept along the dorsal aspect of the sternum while the cutting is done mainly with the ends of the scissors. The bisected sternum is then lifted ventrally with the forceps and the fascia overlying the mediastinum and muscles attached to the sternum is split longitudinally with a new dry swab to expose the thymic lobes. These lobes are freed from surrounding connective tissue by a gentle movement of the swab. The suction cannula connected to a suction flask and vacuum pump, or to a water suction pump is now placed at the anterior end of one thymic lobe and with constant and gentle manipulation of the cannula, the lobe can be aspirated. The same procedure is applied to the other lobe. After removal of both lobes, the area is inspected with the aid of forceps and a swab for possible residual thymic tissue. To close the skin incision, the forearms are released from the rubber band to reduce tension of the skin. Superfine silk with a semi-curved micropoint cutting needle, 6 mm, is used to suture the wound. After suturing the skin, blood stains are removed and the mouse is placed in a warm environment for 30–60 min.

Thymectomy of Mice Aged 2–4 Weeks and Adult Mice

Young animals are anesthetized exclusively with inhalation anesthetics with ethyl ether or methoxyflurane, a derivative of ethyl ether, (Metofane[®], Pitman-Moore, Inc.). Three or more small doses instead of one larger dose of inhalation are required to complete the procedure. Adult mice can be anesthetized with either inhalation or sodium pentobarbital (Nembutal[®], Abbot Laboratories, or Somnopentyl[®], Pitman-Moore, Inc.). Sodium pentobarbital (6mg/ml) is given intraperitoneally at a dose rate 0.1ml/10g of body weight. The mouse is then secured on an operating board with the four extremities fixed with paper adhesive tape. The head is lightly pulled by a rubber band hooked at the upper teeth in order to produce tension at the neck region. The procedure for thymectomy of adult mouse is essentially the same as in the newborn, but binoculars are not used. The thymus can be removed by either suction or the “swab technique”. When the organ is extirpated *by suction*, release of thymic lobes from surrounding tissue by a swab *before* the application of cannula makes the suctioning easier and complete. After the removal of the two lobes, the area is inspected, and the residual thymic tissue, if present, is taken out with forceps and swab. When the thymus is removed *by dowels* thinly-covered with cotton (*swab technique*), the operation is carried out under the lights from a frontal mirror. As shown in Fig. 1,

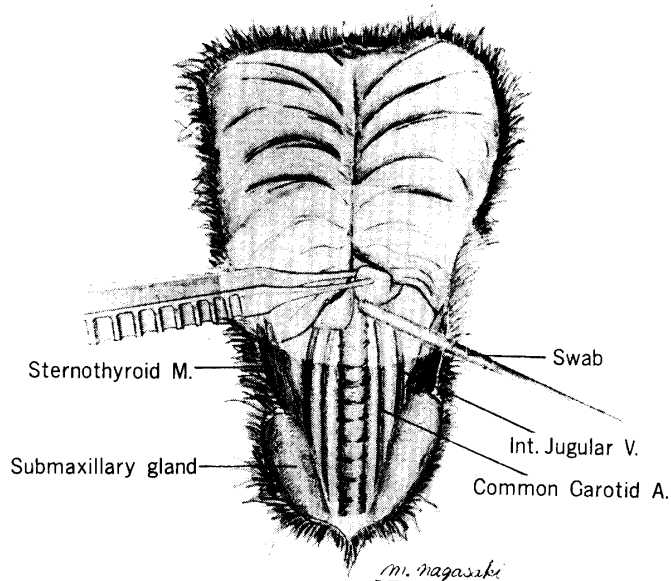


Fig. 1. The thymic lobe is being freed from the surrounding tissue by use of forceps and a swab. Lighting the operating area by use of a frontal mirror makes the tip of the swab and great vessels clearly visible. For illustration, the thymus is over-exposed as compared to the actual operation which is usually done under the bisected sternum.

the thymic lobe is gently detached at the dorsal aspect of the organ by placing and rolling a dry swab, i. e. not wet with blood etc., between the thymus and the trachea. During this procedure, the freed part of thymic lobe can be gently pressed against the chest wall for the prevention of progression of the pneumothorax, if it has occurred, by stopping the air flow into thoracic cavity. After removal of the thymus, one of the forearms is quickly released and then the skin is held together with forceps. By topical application of cyanoacrylate glue (Alon-alpha[®], Toa Chemicals) the wound is closed air tight. In the case of pneumothorax, before glueing but after closure of the skin, part of the trapped air can be forced to bubble out of the thorax by slight pressure with the fingers towards the chest wall. This helps to reduce ill effects of the pneumothorax on the recovery of the mouse. The mouse is removed from the operating board and placed in a warm chamber for full recovery from anesthetic.

RESULTS AND DISCUSSION

We used superfine silk for suturing the skin of the newborn mice in order not to unduly arouse the interest of the mother mouse and subsequently avoided destruction of these animals by the mother, a major cause of loss of these mice. These baby mice heretofore survived the surgery at a rate of 60–70% even when the operator had no previous experience. This rate went up to 80–90% when the operator was experienced, that is after the performance of about 50 operations.

In the case of adult mice, control of pneumothorax, which is not an uncommon complication, and prevention of massive bleeding, which is fatal, are key factors. Use of suction is an advantage in the speedy operation, but this often results in an incomplete removal of the thymus, partly because of the use of a cannula of improper size. The problem was solved by utilizing swabs to detach the thymus from surrounding tissue, as much as possible, before a suction cannula was applied. Of the 500 mice on which this procedure was used, the mortality rate was less than 10%.

When the thymectomy was done by "swab technique", use of a head mirror was more advantageous over other lighting systems. The swab in relation to the great vessels is directly visible by the operator, and such is impossible using ordinary illumination. This greatly reduces the chance of damaging vessels which in turn induces a massive hemorrhage. This also delayed the onset of pneumothorax or totally avoided a pneumothorax as unnecessary movements of the swab were eliminated. This technique was also effectively applied in the case of older mice in which the thymus was involuted and more firmly attached to the surrounding tissue.

All mice thymectomized were examined for thymus remnants at the end of experiments. The incidence of remnants was less than 1% out of approximately 1,000 mice.

In any thymectomy procedure (1-4) experience is the main prerequisite for a successful operation. We consider the practice of the thymectomy with swab to be essential for a beginner because proper handling of the organ, that is how deep one may go into the mediastinum without causing pneumothorax, how fast one has to close the skin after the onset of pneumothorax and how to avoid massive bleeding, can all be learned.

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