

Technique for Hypophysectomy of the Mouse¹

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SUMMARY

A procedure for hypophysectomy in the mouse by a parapharyngeal approach is described. The technique is simple and applicable to mice of any age; the mortality is low. No additional therapy was required for BALB/c mice, but the animals do better if given glucose in

saline after the operation. The completeness of the operation was checked by observation of the weight loss and by gross inspection and histologic study of the pituitary area.—*J Nat Cancer Inst* 30: 1077-1089, 1963.

HYPOPHYSECTOMIZED MICE have been used in hormonal research since 1933. In that year Selye, Collip, and Thomson (1) reported successful removal of the pituitary gland from female albino mice. They used Smith's procedure of hypophysectomy previously described for the rat (2). This involved a parapharyngeal approach to the base of the skull. A dental burr was used to drill the bone opposite the pituitary fossa, and suction was used for the removal of the pituitary gland. The disadvantages of Smith's procedure when first applied to mice were: (a) high mortality from hemorrhage and injuries associated with drilling; (b) difficulty of complete removal of the flat, elongated pituitary of the mouse through a rounded hole smaller than the gland itself.

In 1938 Thomas (3) used the same parapharyngeal approach as Smith, but instead of drilling, he removed a quadrilateral panel of bone from the basiocciput.

Thomas's operation had the advantage of total exposure of the pituitary gland and its removal under direct vision. He also claimed a lower mortality when young mice were used. The disadvantages of Thomas's procedure are the technical difficulty and high mortality experienced when old mice weighing more than 20 g were used. In such animals the

¹ Received January 23, 1963.

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³ I am grateful to Dr. Thelma B. Dunn for her professional guidance, Mr. G. Gsell for preparing the photomicrographs, Mr. B. Baker for the illustrations, and Mr. A. Green, Mr. E. J. Soban, and Mr. H. Neil Deese for their technical assistance.

⁴ National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

bone is dense, and the spheno-occipital synchondrosis is tough and narrow. Therefore, it is difficult to remove the bone flap without troublesome hemorrhage. Also, in some strains of mice such exposure posterior to the synchondrosis may not allow a direct view of the whole pituitary. Another disadvantage is that the operation has to be interrupted from time to time to allow the animal to breathe.

Lostroh (4) used the technique of Smith on 83 animals of 5 different strains of mice. She devised tracheal cannulation to avoid interrupting the hypophysectomy, and described special postoperative care including resuscitation in oxygen and hydrocortisone administration for strain A/He mice. Serial sections of the pituitary gland region were not made to check the completeness of removal.

Young (5) modified Thomas's technique by removing the synchondrosis along with the basioccipital flap of bone for a more complete view of the pituitary gland. He used this method with 432 mice and determined the frequency of remnants by serial sections.

I attempted to destroy the hypophysis in BALB/c mice by injecting absolute alcohol or Zenker's solution, but the results were unsatisfactory.

The procedure for hypophysectomy of the mouse to be described is a modification of the technique of Smith. Mice hypophysectomized by this method have been used to study the growth of an adrenocortical tumor in strain BALB/c, and the same approach was employed to introduce carcinogens into the pituitary gland.

MATERIALS AND METHODS

Instruments.—1) Board with attached rubber bands to secure the legs of the mouse and a transverse wire fixed in two corks to immobilize the head when the upper incisors were held beneath the wire. 2) Small self-retaining retractor. 3) Curved sharp needle. 4) Curved forceps. 5) Cotton pledgets (made by winding small pieces of cotton around the pointed end of toothpicks). 6) Discoid dental scraper. 7) Dental drills Nos. 3 and 5. 8) A glass cannula, 12 cm long, with a tip drawn to about 0.65 mm in diameter. The cannula was curved at about a 125° angle, 2.5 cm from the tip. This was attached to a rubber tube about 20 inches long. This device was used to remove the pituitary gland by oral suction. A properly adjusted vacuum pump could be used instead. 9) Straight surgical needle and silk sutures (Nos. 4-0). 10) Optical equipment: The operation was performed under a dissecting binocular (objective No. 1 eye piece, $\times 10$). This allowed for a working distance of 3.5 inches. Since the approach is deep and narrow, strong light was reflected in a downward and forward direction by a mirror fixed in the center of the binocular (fig. 1a).

Anesthesia.—Mice received intraperitoneal injections of 60 mg per ml of veterinary Nembutal that was supplied in liquid form (Abbott) and diluted 1 part in 10 with saline or distilled water. The solution was always

freshly prepared because it deteriorates after 48 hours. The diluted solution was injected intraperitoneally according to body weight (table 1). With this dose, anesthesia is accomplished after 4 to 5 minutes and lasts about 15 to 20 minutes. If too deep anesthesia is induced, the mouse will aspirate blood into the lungs during the recovery period.

TABLE 1.—Dose of Nembutal anesthesia according to body weight of mouse (Nembutal was diluted $\frac{1}{10}$)

Body weight (g)	Solution (ml)	Body weight (g)	Solution (ml)	Body weight (g)	Solution (ml)
10	0.14	17	0.21	24	0.28
11	0.15	18	0.22	25	0.29
12	0.16	19	0.23	26	0.30
13	0.17	20	0.24	27	0.32
14	0.18	21	0.25	28	0.34
15	0.19	22	0.26	29	0.35
16	0.20	23	0.27	30	0.37

Operative technique.—The ventral neck region was shaved, and the mouse was fixed on the board in a dorsal position with its head away from the operator.

A midline incision, 1.5 cm long, was made from the chin to the sternum. The salivary glands were separated in the midline and retracted laterally by a small self-retaining retractor (fig. 1b). The pretracheal muscles were split in the midline with the sharp curved needle (fig. 1c), and the left pretracheal muscle was cut through its upper third (fig. 1d) and retracted downward to expose the underlying structures. A small hole was made in the lower part of the trachea with the curved needle, so that the mouse could breathe through this hole during the next steps of the operation. The landmark for approach to the base of the skull was the left longus capitis muscle as it appears below the level of the thyroid gland, between the trachea medially and the left carotid artery laterally (fig. 1e). A curved forceps was introduced in this space, pulled upward, and then opened carefully so that the tracheal hole was not obstructed. This retraction revealed the two longus capitis muscles separated by a vertical midline crest and converging upward to the spheno-occipital synchondrosis. A better exposure of the bone around the synchondrosis was achieved by removal of the pharyngeal mucous membrane from the bone in a cranial direction and separation of the longus capitis from occiput in a caudal direction. A discoid dental scraper was used in this step, and care was taken not to injure the internal carotid arteries on either side (fig. 1f). It is extremely important to place the drill hole exactly in the midline, and the best landmark of the midline is the vertical occipital crest. The cephalic-caudal placement of the drill hole has to be centered on the synchondrosis (fig. 1g). If drilling is made too far forward, the transverse venous canal will be opened; if too far caudal, removal of the pituitary gland is difficult and injury of the medulla is likely. The dural sheath is usually also opened during drilling of bone, and the pituitary gland can be seen bulging into the hole in the bone. The pituitary

was sucked out with the glass cannula by suction, applied mainly in a lateral direction (figs. 1h and i). When removal of the pituitary was complete, the sellar diaphragm could be seen as an intact transparent membrane. A slight amount of bleeding occurs during drilling or after suction. In young mice this may be troublesome, but we controlled this by placing a pledget over the drill hole and leaving it undisturbed for 2 to 3 minutes. The skin was then closed with 2 interrupted silk sutures. The whole operation takes from 4 to 6 minutes. Recovery was complete after 15 to 20 minutes. This technique was successfully used on 160 mice.

The advantages of the present technique are:

- 1) Applicability to mice of any age.
- 2) Simplicity of approach to skull base by cutting the left pretracheal muscle.
- 3) Tracheal breathing hole to avoid asphyxia of the animal or interruption of operation.
- 4) Low mortality during and immediately after operation. In the last series of 50 mice, none were lost during the surgical procedure or in the immediate postoperative period.

Postoperative care.—The mice were maintained on Purina laboratory chow and given 5 percent glucose in normal saline *ad libitum* instead of drinking water. Weights were recorded daily for 2 weeks and then weekly until the mice were killed. The mice survived better if kept at a temperature of 80 to 83° F. Some hypophysectomized mice could be kept alive for 2 months without any replacement hormonal therapy. About 10 percent of the mice died after the first week; these previously healthy animals became extremely wasted and then paralytic before death. Replacement therapy was not tried; these mice might have survived if given cortisone.

Test of completeness of operation.—Female BALB/c mice between 26 and 83 days old were used. Body weight at operation ranged from 11 to 23 g.

After the mice were killed by ether, the hypophyseal fossa was examined with a binocular microscope to see if any pituitary remnants could be recognized. The adrenal glands and female genital organs were examined for evidence of atrophy. After fixation in Zenker-formol solution the skulls were decalcified by the method of Morse (6). Each skull was trimmed coronally before and behind the pituitary fossa. Paraffin sections were cut in a coronal plane, 6 μ thick. The pituitary fossa was examined at intervals of 30 μ , which resulted in about 60 sections for each mouse. Sixty-five mice were examined in this way.

RESULTS

Figure 2 shows the anatomical orientation and histologic pattern of the pituitary gland. In coronal sections most of the anterior pituitary lies laterally, while the pars intermedia and pars posterior lie centrally and are separated from the pars anterior by a cleft. Histologically the

TABLE 2.—Frequency of pituitary gland remnants in 65 mice

Remnants	Size of remnants*	Number of mice	Percentage of total
Anterior pituitary	—	38	58
	+	8	12
	++	11	17
	+++	8	12
Posterior pituitary	Present	4	6
	Absent	61	94

*See first paragraph under Results for explanation of symbols.

anterior pituitary is easily distinguished because of its characteristic cells and pattern, and any remnants are easily recognized. Most of the remnants were from the anterior pituitary and were located laterally. Remnants of the posterior pituitary were found in only 4 mice and were located in the midline. Table 2 shows the size and frequency of anterior pituitary remnants. The subjective grading of Young in 1955 (5) was used to express the size of remnants as follows:

— = no remnants (fig. 3); + = fragments composed of few cells with acidophils, viable or degenerate (figs. 4a and b); ++ = fragments large enough for the typical histologic pattern to be recognized (fig. 5); +++ = large fragments accounting for 10 percent of the gland or more (fig. 6).

Most remnants of grade (+) were degenerate and probably nonfunctioning. Almost all mice with remnants (+++) were from the first group we did. Infection of the air sinuses was common in hypophysectomized mice as compared to intact (figs. 3 and 5).

Table 3 shows a comparison of gross inspection and histologic study of the sella for pituitary gland remnants. This indicates that gross inspection alone is unreliable. Some apparent remnants seen on gross inspection proved to be an organized blood clot on histologic study (fig. 7).

TABLE 3.—Comparison of gross inspection and histologic study for detection of pituitary gland remnants

Number of mice	Macroscopic remnants*	Histologic remnants*
29	—	—
21	+	+
10	—	+
5	+	—
Total 65		

*See first paragraph under Results for explanation of symbols.

DISCUSSION

It is important for those using hypophysectomized animals to know whether removal of the pituitary gland is complete. A detailed comparison of the various criteria of hypophysectomy is described by Young and

Fraser (7). Pituitary remnants can be forecast by the observation of body weight in the first few postoperative days. For practical purposes, if the weight of any mouse exceeds the preoperative weight by 10 percent or more by the 7th postoperative day, then it is almost certain that functioning pituitary tissue is left. The advantage of determining the completeness of hypophysectomy by weight is that this criterion is simple and the method does not disturb the mouse. The disadvantage of this method is that completely hypophysectomized animals may occasionally gain weight and mice may lose weight from causes other than hypophysectomy. Griffiths (8) and Lostroh (4) used the criteria of atrophic changes in target organs and the absence of macroscopic pituitary fragments.

Bahner and Von Graff (9) considered that remnants of pituitary gland can be reliably indicated by gonadal weight, particularly seminal vesicle weight. The study of remnants by serial sections of the pituitary area is laborious and time-consuming and does not reveal the functional activity of the remnants. It is noteworthy that remnants of size + are biologically nonfunctioning and do not show evidence of regeneration even 3 months after operation (Young).

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PLATES

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FIGURE 1a.—Optical equipment for hypophysectomy of mouse.

1b.—Position of animal and retraction of ventral neck incision.

1c.—Separation of pretracheal muscles at midline.

1d.—Cutting the left pretracheal muscle at its upper third.

1e.—Area of approach to the base of skull.

1f.—Exposure of base of skull showing landmark of midline (occipital crest) and site of drilling (synchondrosis).

1g.—Drilling.

1h AND 1i.—Suction of pituitary gland.

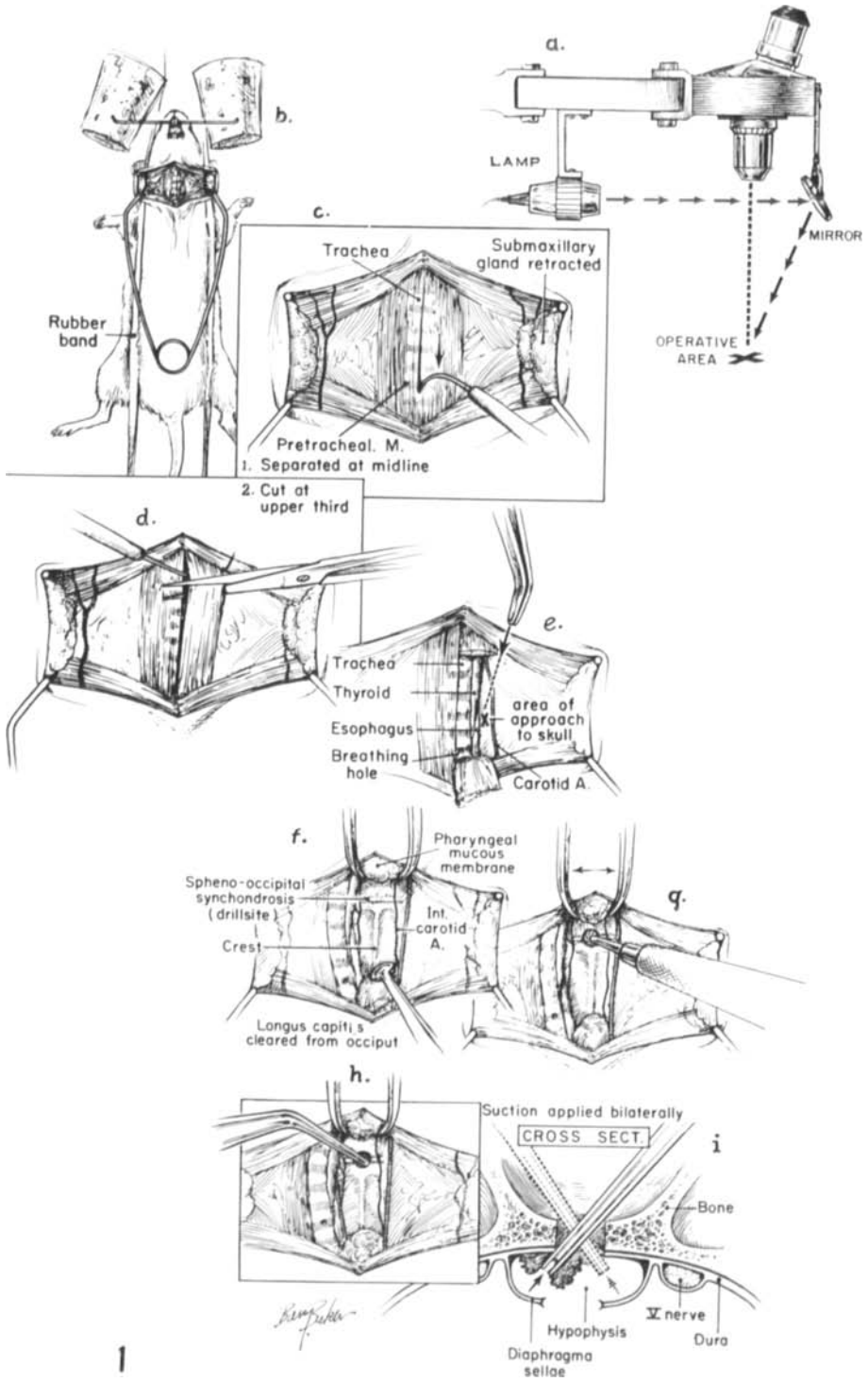


PLATE 191

FIGURE 2.—Anatomical orientation of pituitary gland in mice. In coronal section the anterior pituitary is mostly laterally placed. Hematoxylin and eosin. $\times 33$

FIGURE 3.—Complete hypophysectomy showing intact sellar diaphragm. *Note* sinus infection. Hematoxylin and eosin. $\times 33$

FIGURE 4a.—Degenerate microscopic remnants (+) of anterior pituitary. $\times 290$

FIGURE 4b.—Microscopic remnants (+) of anterior pituitary. $\times 420$

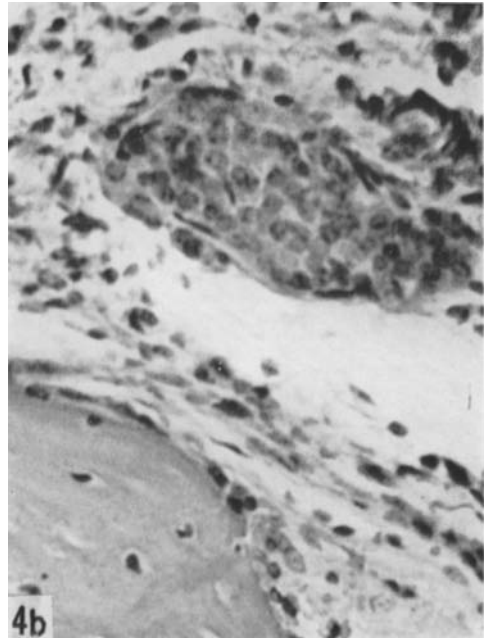
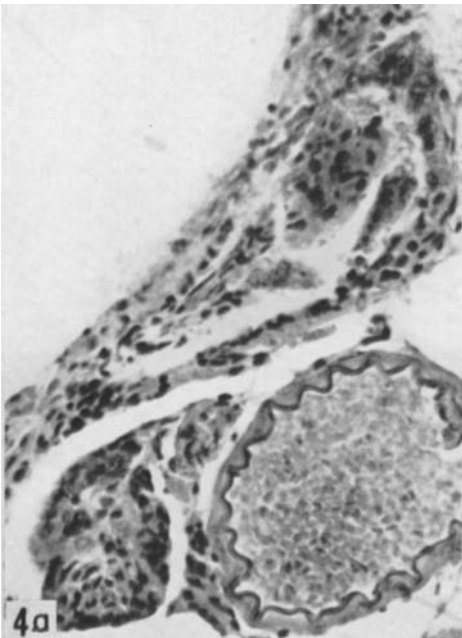
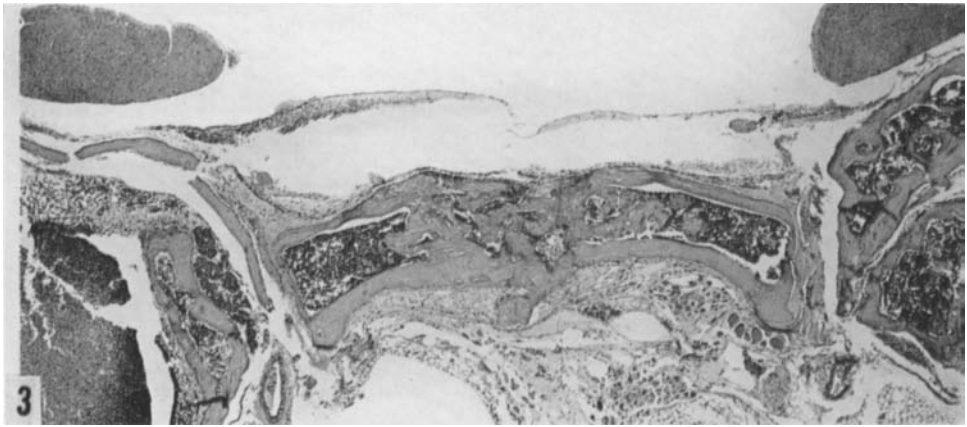
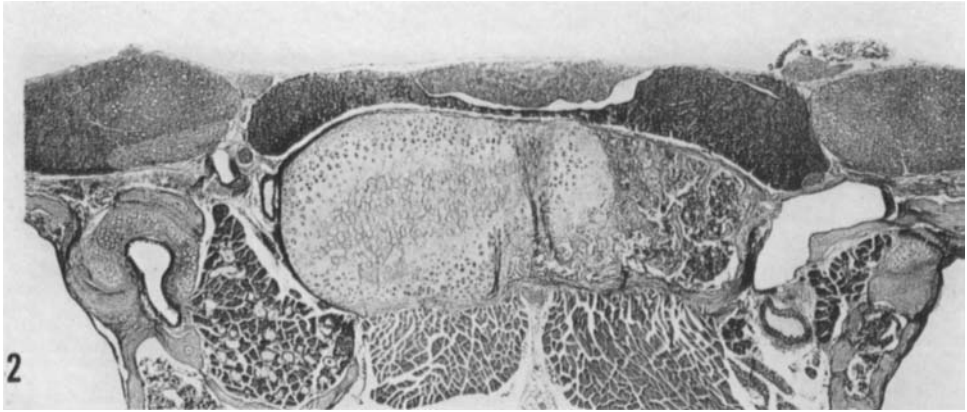


PLATE 192

FIGURE 5.—Remnant of anterior pituitary gland of mouse (++) . Hematoxylin and eosin. $\times 33$

FIGURE 6.—Remnant of anterior pituitary (+++) partly ossified. $\times 33$

FIGURE 7.—Organized blood clot mistaken grossly as remnant. $\times 290$

