isolated from the stools of HF patients and from the soil of the endemic area in Korea.

Summary. Sera from 17 patients convalescing from hemorrhagic fever and 50 normal controls were tested for the presence of neutralizing antibodies of *Cl. perfringens* iota toxin. Hemorrhagic fever sera contained a significantly higher quantity of neutralizing antibodies than did the control sera.

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Improved Procedure for Hypophysectomy of the Mouse.* (22004)

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As early as 1933, Selye, Collip and Thomson(1) reported the successful removal of the pituitary from female albino mice; no data on survival were included, although it was stated that mice hypophysectomized by the parapharyngeal approach tolerate the operation better than do rats. Four years later, Leblond and Nelson(2) published an extensive report on the histological changes observed in the adrenal, thyroid, and gonads of mice of the A and CBAN strains following hypophysectomy; a high mortality during the first postoperative week was reported, although the animals that survived this critical period subsisted thereafter apparently with no special therapy. Subsequently, Thomas(3) described a technic for which he used 2 minute hooks in place of the more customary dental drill to break through the cranium. The procedure for mouse hypophysectomy described in the present communication utilizes the basic technic of Smith(4), but introduces modifications which insure completeness of operation and make possible an operative survival approaching 100%.

Materials and methods. Materials. A cork-topped board of convenient size is outfitted with 2 rubber bands held taut by thumbtacks in order to secure the limbs. For immobilizing the head, a malleable wire is stuck firmly into the board and bent so that the incisors of the animal can be hooked securely beneath it. A 20-gauge one-inch injection needle bent at an angle of 120° serves admirably as a tracheal cannula. Cotton pledgets or miniature swabs are made by winding a small piece of cotton around the end of a pointed toothpick. These materials, together with a view of the operative setup, may be seen in Fig. 1. Other necessary equipment, forceps, dental drill, No. 5 dental burr, binocular magnifier mounted on a stand, and adjustable lighting, are similar to those required for the operation in the rat (5). Nembutal is used for anesthesia. 850 mg of sodium pentobarbital (USP) dissolved in 4 ml of propylene glycol, 2 ml of ethanol, and 14 ml of water serves as a stable stock

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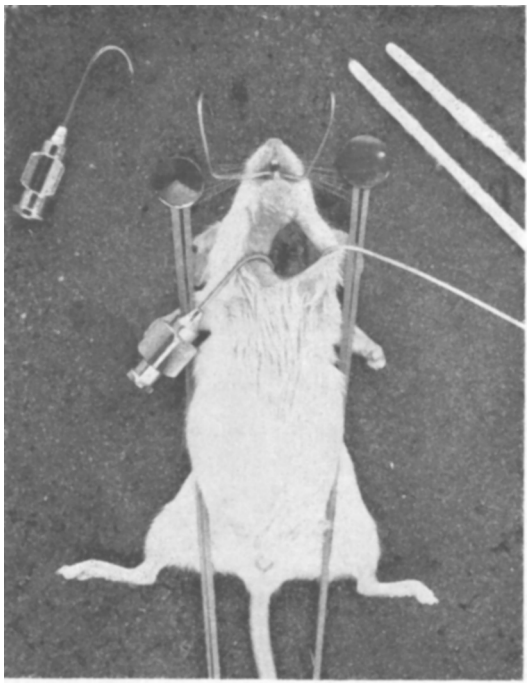

FIG. 1. Operative setup for hypophysectomy of the mouse.

solution.† After the concentrated solution is diluted with distilled H_2O (1:4), injections are administered in a dose of 0.01 ml (0.085 mg of sodium pentobarbital) per g of body weight. *Operative technic.* Just before operation, the mouse is injected intraperitoneally first with the anesthetic agent and then with 0.25 mg of cortisone. The ventral neck region is shaved, a longitudinal midline incision, measuring 1 cm, is made, extending downward from the center of the lower jaw, and the sternohyoid muscle is separated so that the curved injection needle can be hooked gently into the trachea. Following cannulation, the base of the skull is reached by the usual parapharyngeal approach, as described for the rat(5). In the mouse, special care must be taken to distinguish between the synchondrosis itself and the anteriorly located transverse venous sinus. For the best exposure of the underlying pituitary, the hole drilled into the cranium should be so placed that one-third is above and two-thirds are below the occipito-sphenoidal synchondrosis. The intact lobes are then gently aspirated from the hypophyseal fossa; strong suction must be avoided since it ruptures the protective membrane which separates the pituitary from the brain proper. Upon removal of the gland, a pledget is inserted into the hole and the skin is closed with a single suture. Bleeding is usually negligible, and the pledget is removed before the animal is resuscitated. *Postoperative care.* Immediately following operation, the animal is placed in an atmosphere of 95% O_2-5% CO_2 until it recovers. It is then injected intraperitoneally with 1 mg of terramycin dissolved in 1 ml of 5% glucose solution. A similar injection is given on the second postoperative day along with a subcutaneous injection of 0.25 mg of hydrocortisone. Most strains require no additional therapy. For the best assurance of survival, hypophysectomized mice should be kept in a room free from drafts, at a constant temperature of approximately 82° F. *Completeness of hypophysectomy.* Immature female mice of the BALB/c, A/He, C_{57}, C3H and Swiss albino strains were hypophysectomized at 35 days of age by the technic described above. One month later they were sacrificed and their adrenals, ovaries and uteri were examined by gross observation for evidence of atrophy. These organs were then removed, dissected free from surrounding tissue and weighed on a Roller-Smith torsion balance. The non-operated animals were treated in a similar manner.

The *results* are summarized in Table I. At the time of autopsy, the hypophyseal fossa was also examined with a binocular microscope for possible pituitary remnants. Serial histological sections of the region were not made, since nearly all the operated animals showed marked atrophy of the organs associated with hypophyseal function.

Discussion. The method outlined herein is a modification of the technic developed by Smith(4) for hypophysectomy of the rat. It has been used with success on 5 strains of mice whose body weight is not less than 12 g. In smaller animals, difficulties in cannulation, and bleeding from the cranium, have been encountered. Some workers prefer to dispense with tracheal cannulation and to

† The commercial solution of nembutal was found to be unsatisfactory.

TABLE I. Effects of Hypophysectomy on Female Mice Operated on at 35 Days.

Strain	Operative condition*	No. of animals	Body wt, g: Day of operation	Body wt, g: At autopsy	Organ wt, mg: Adrenal	Organ wt, mg: Ovary	Organ wt, mg: Uterus	Width of tibial epiphyseal cartilage plate, μ
BALB/c	N†	10	15	19	5.4 ± .2‡	5.1 ± .04	45.7 ± 9.3	103.5 ± 2.2
	H	8	16	13	2.1 ± .2	1.1 ± .07	5.9 ± .6	71.2 ± 1.5
A/He	N	11	15	20	3.9 ± .2	4.7 ± .3	51.5 ±28.4	120.8 ± 5.9
	H	10	16	13	1.5 ± .1	1.1 ± .06	5.3 ± .7	60.2 ± 1.1
C57	N	10	13	19	3.9 ± .2	6.4 ± .3	65.5 ± 3.2	119.2 ± 1.7
	H	8	15	14	1.5 ± .1	1.1 ± .1	4.9 ± .5	48.5 ± 2.3
C3H	N	7	18	23	4.0 ± .2	10.1 ± .2	85.1 ± 9.1	119.8 ± 2.8
	H	10	18	15	1.6 ± .2	1.2 ± .1	6.1 ± .6	58.0 ± 1.1
Swiss albino	N	4	19	23	8.7 ± .01	7.0 ±1.6	50.1 ± 8.7	123.2 ± 2.6
	H	5	22	19	2.7 ± .4	2.2 ± .3	10.2 ± 1.3	60.7 ± 2.1

* All animals operated on at 35 days of age and sacrificed at 65 days.
† N = Nonoperated. H = Hypophysectomized.
‡ Mean ± stand. error.

permit the animal to breathe intermittently; however, it has been our experience that the attendant manipulation precipitates rupture of the delicate blood vessels.

All mice of the different strains after hypophysectomy show similar retardation of general body growth, inhibition of skeletal growth, and a marked degree of atrophy of the adrenals, ovaries and uteri. However, strain differences do become apparent during the postoperative period. Mice of the C3H and A/He strains require resuscitation in an oxygen-CO_2 atmosphere if better than 50% survival is to be expected. In addition, the latter strain must be supplied with an adrenal steroid following the operation and at regular intervals thereafter. 50% of the hypophysectomized females of the A/He strain not injected with hydrocortisone died within the first 24 hours and 80% were dead within 48 hours, whereas none of the hypophysectomized mice treated with hydrocortisone succumbed; 0.25 mg of hydrocortisone given subcutaneously as a saline suspension every tenth day was found to be sufficient to maintain this sensitive strain. C57BL, C3H, BALB/c and Swiss albino mice seem to survive indefinitely without further hormonal injections.

Summary. A practical procedure for mouse hypophysectomy by the parapharyngeal approach has been developed. Innovations include tracheal cannulation, resuscitation in an O_2 atmosphere, and hydrocortisone therapy. Mice of the A/He strain are particularly vulnerable to the effects of pituitary loss and will survive only if they are supplied with an adrenal steroid at regular intervals. On the other hand, mice of the C57BL, C3H, BALB/c and Swiss albino strains do not require special postoperative handling.

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