

6. K. Diem and C. Lentner, *Documenta Geigy Scientific Tables* (CIBA-GEIGY Ltd., Basle, Switzerland, ed. 7, 1970), p. 709.
7. N. Cohen, H. B. Spitz, M. E. Wrenn, *Health Phys.* **33**, 431 (1977).
8. R. D. Lloyd, C. W. Mays, G. N. Taylor, D. R. Atherton *ibid.* **18**, 149 (1970).
9. For the adolescent, the skull extrapolation technique did not agree with a balance sheet method (that is, total body = liver + lungs + skeleton). Since maximum confidence was placed in the whole-body measurement, and since bone

growth was rapid in the youth, the latter method of calculation was used to obtain his skeletal estimates.

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## Neonatal Rat Surgery: Avoiding Maternal Cannibalism

**Abstract.** *A simple program of handling and care of pregnant rats before delivery makes it possible to carry out surgical procedures on newborn pups without resultant cannibalism or rejection of the operated animals by their mothers.*

Newborn rats subjected to surgical procedures and returned to their mothers are often killed and eaten by the mothers. Frequently, merely handling pups, as in the carrying out of simple injection procedures, produces similar results, or the young animals may be ignored and left to die. Here we describe methods for manipulating mother and young so as to obviate these undesirable occurrences. These methods have enabled us to carry out surgical procedures with considerable tissue removal and blood loss upon rat pups born only several hours before surgery. Maternal cannibalism and abandonment have been extremely rare, even when all pups in a litter have undergone surgery. Moreover, the operated animals gained weight and developed normally. These methods may be applicable to many other small mammals and may thereby greatly extend the utility of these organisms in studies in which surgery in early life is required.

**Preparation of the mother.** Whatever the source of the pregnant rats, they must be placed in breeding cages by the 13th day of gestation. We house commercially obtained gravid females in separate stainless steel cages approximately 30 by 25 by 61 cm. The cage must be large enough to accommodate the parent, the litter, food pellets, a thick layer of wood shavings, and the excreta that accumulate between confinement and weaning some 30 days later, because during that period the cage may not be cleaned. The cages are placed in dimly lit areas of the laboratory and covered from above with a cloth towel to further reduce the light level within. Often the maternal parent will draw a corner of the towel through the cage-top louvers and shred it for use as nest material. Each towel and water bottle should be assigned to an individual cage.

Starting on the day after the gravid females are installed in individual cages,

and each day thereafter until the pups are delivered, the surgeon must follow a procedure that we have termed "hand gentling." This consists of removing the covering towel and cage top and placing one's hand in the cage near but not touching the female. In response to this incursion, and generally in a single motion, the rat will move its head so as to inspect the hand with its vibrissae and nose. After this has occurred the surgeon touches and, if possible, strokes the rat. It is helpful to repeatedly present the hand for nasal inspection during this ritual. With each successive day before birth, the surgeon extends the length of the gentling process, so that by day 20 of gestation there are 3 to 5 minutes of such contact. Simultaneously, the complexity of the encounter is increased, incorporating those maneuvers which later will be used to remove and handle the pups.

On day 17 of gestation several paper towels are placed inside the cage to assure the availability of nest-building materials. At this time the nursery area is darkened further so that little light reaches the cage floors. On day 20 of gestation noise in the immediate and the surrounding areas is reduced, and on the following day entry of humans other than the surgeon is restricted.

Employing these precautions we have been able to accommodate as many as 12 litters simultaneously in a 4.2 by 3 m room while still using its entire bench-top area for daily activities. Although predation and maternal rejection were rarely noted after surgery, it is advisable to breed several reserve litters in order that wet nurses be available should the mother die or fail to produce an adequate quantity of milk.

**Presurgical and surgical care of pups.** Generally, to effect adequate analgesia, we have employed hypothermic exposure by placing pups in a cold room. In our experience barbiturate or ether administration has somewhat unpredict-

able effects upon such young rats. When pups are placed in an environment of 7°C they become torpid within 30 to 45 minutes and also display reduced blood loss during surgery. The hands of the surgeon, however, warm the pup, and the torpor is dissipated within 5 to 7 minutes. This timing may be advantageous, permitting completion of relatively brief surgical procedures and return of the pup to the nest as it awakens. The awakened pup will often crawl to the teat and commence suckling immediately.

**Maternal response to repeated intrusion.** We have frequently employed rat pups 1 to 5 days old in studies of limb regeneration after above-elbow amputation of a forelimb. After surgery each pup was immediately returned to the nest area, was accepted by its parent, and commenced nursing forthwith. When a cage top is opened repeatedly as one pup after another is removed and then replaced, the mother may take a pup in its mouth and move some distance away from the nest and then extend its neck so that jaws and pup are thrust upward. Frequently this is accompanied by exaggerated running, purposeless changes of direction or digging maneuvers, and sometimes by return of the pup to the nest and replacement with another. We view these actions to reflect considerable distress of the mother and immediately discontinue our work with the pups. Often further hand gentling calms the mother and then manipulation of the pups may be safely continued. At other times it is useful to cover the head of the mother with a cage-top towel or to fold it so that it can be used as a cage divider separating the mother from the pups while at the same time blocking the nest from its line of sight. This last maneuver is particularly useful when members of the litter are to receive injections and the absence of the mother from the nest for several minutes is desired.

It is essential to ascertain that the pups are being fed by the mother. It does not suffice that they are suckling. During the first week of life, the pups should be lifted from the mother daily and their bellies inspected. Well-fed pups display distended abdomens. The whitish milk-filled stomach can be seen beneath the liver through the thickness of the abdominal wall.

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### Notes

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