same intensity (6) and within the range observed for *G. integer*. Both sounds were played simultaneously for 1-minute periods, and information was recorded on the time from the start of a trial until the fly reached a speaker and the total time (7) spent by a fly on each speaker (Table 1).

After approaching a cricket mounted on a speaker, a fly would always walk quickly around and on the cricket (Fig. 1). After each trial I removed the fly, speaker, and cricket from the box, and in every case I found fly larvae on the cricket and on the surface of the speaker. Larvae on a cricket crawled across the surface of the cricket and burrowed into it.

Larvae deposited on the surface of a speaker stood on their posterior ends and waved the pointed anterior ends in the air. These larvae easily adhered to any surface with which they were brought into contact. I observed a live tethered cricket walking across a speaker containing fly larvae. I then examined the cricket and found several larvae attached. By depositing larvae in the area around a singing cricket, a fly might parasitize other crickets attracted by that cricket's song.

Sabrosky (8) reviewed the evidence on host relationships in *Euphasiopteryx* and concluded that these flies probably parasitize nocturnal Orthoptera (9). I infected 40 crickets by placing on them larvae that had already been deposited by the flies. These crickets produced 28 pupae from which 11 adult flies were reared (10).

While two flies were in the acoustical box, the song of another cricket species was played (11). Both flies were attracted to this cricket song, an indication that this fly has not specialized on a single cricket species. Supporting this hypothesis, Walker and Mangold (12) have recently observed the attraction of *E. ochracea* to the tape-recorded song of the mole cricket *Scapteriscus acletus*, and have raised adult flies from artificially infected *S. acletus* and *Gryllus rubens* males in Florida.

Walker (1) discussed the evolutionary aspects of acoustically orienting predators. He suggested that song characteristics such as irregularity, duration, time of singing, and others could be influenced by selective pressures resulting from acoustically orienting predators. Walker (13) also suggested that such pressures may be responsible in part for the occurrence of noncalling cricket species. Acoustically orienting parasites should also produce counteradaptations in their hosts.

The presence of acoustically orienting flies may influence the behavior of *G. integer* males observed in the field (14). For most of the night, cricket aggregations are composed of calling as well as noncalling males. Noncalling males, termed satellites, walk in the area occupied by calling males and attempt to intercept and copulate with females attracted by the calling males. In the hours just before sunrise, the number of singers increases as some satellites begin to sing.

Table 1. Fly phonotaxis within an acoustical box (7).

Fly No.	Time to first speaker (seconds)	Total time on speakers (seconds)	
		Cricket song	Control sound
1	8	34	0
2	5	21	0
3		0	0
4	4	16	0
5	11	27	0
6	14	23	0
7	8	27	0
8	3	20	0
9	5	48	0
10	12	18	0

Flies are attracted to taped cricket song during the hours when some satellites sing. I collected calling and noncalling male crickets and examined them for fly larvae. Of 11 calling males 9 had larvae deposited on them, whereas only 1 of 17 noncalling males had been parasitized (χ^2 = 16.8, P = < .002). Satellite males apparently experience a lower incidence of parasitism by not singing, at least for a portion of the night. Soper *et al.* (15) describe *Colcondamyia auditrix*, a previously unidentified species of sarcophagid fly, and note that this fly orients acoustically to cicada song. Male cicadas, once parasitized, are rendered incapable of producing song; as a result, multiple parasitism is limited and parasitized cicadas are prevented from attracting females acoustically. Acoustically orienting parasites probably exert selective pressures on male reproductive behavior in many animals.

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1. T. J. Walker, *Fla. Entomol.* **47**, 163 (1964). The singing prey in these experiments were crickets.
2. Cricket song was produced by Uher 4000 Report-L tape recorders; Realistic model 40-1228 speakers were used in the field, and model 40-1224 speakers in the laboratory.
3. Fly traps consisting of aluminum screen coverings with funnels projecting inward were fitted to each speaker during 1975.
4. Living crickets were not used because they would move around on the surface of the speaker.
5. Control sounds were a high-frequency whistle for the first five trials and a bullfrog croaking for the second five trials.
6. Sound intensities were measured with a General Radio model 1565-B sound level meter held 6 cm from the speaker.
7. The total time spent by a fly on the speaker from which the cricket song was emanating was usually the result of two or more visits to the speaker.
8. C. W. Sabrosky, *Proc. Entomol. Soc. Wash.* **55**, 167 (1953).
9. The insectan order Orthoptera includes crickets, katydids, and their relatives.
10. Parasitized crickets always died just before or after larval emergence.
11. The song of a tree cricket was used.
12. T. J. Walker and J. Mangold, in preparation.
13. T. J. Walker, *Fla. Entomol.* **57**, 13 (1974).
14. W. Cade, in preparation.
15. R. S. Soper, G. E. Shewell, D. Tyrrell, *Can. Entomol.*, in press. This reference was brought to my attention after this report was submitted for publication.
16. I thank D. Otte for his guidance and assistance, R. Gagne and L. Knutson for identifying the fly, L. E. Gilbert for photographing the fly, and T. J. Walker who alerted me to the existence of an unpublished reference (15). The acoustical box was built by the late J. Loftus-Hills.

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Weaning and Growth of Artificially Reared Rats

Abstract. *The importance of suckling experience for later feeding in the rat was tested by means of an isolate rearing technique that eliminated oral feeding. Pups reared in the nearly complete absence of suckling and feeding ate and drank at weaning and then grew normally. Furthermore, the characteristics of apparently normal ingestion and growth make the artificially reared rat a useful preparation for other developmental investigations.*

Despite demonstrations that characteristics of mothers' milk can influence the food preferences of weanling rats (1), the importance of the preweaning suckling experience for the normal development of feeding is not known. Does adult ingestion emerge at weaning, independent of nursing experience; or must the pup participate in the behavioral transitions of suckling (2) and weaning (3) in order that normal feeding occur? An understanding of the contribution of suckling to later feeding has been hampered by the technical difficulty in manipulating suckling experience. Using a simple and practical technique for directly assessing this contribution, I now report that rats reared in the nearly complete absence of suckling and feeding are indistinguishable from normally reared siblings in their initial responses to food at weaning and in their postweaning growth. The feeding experiences of the suckling period, therefore, do not appear necessary for food recognition and ingestion at weaning, or for near normal growth after weaning.

Previously reported methods for hand-rearing rat pups (4, 5) were not suitable for this investigation because these hand-feeding techniques provide pups with oral experience which may contribute to learning

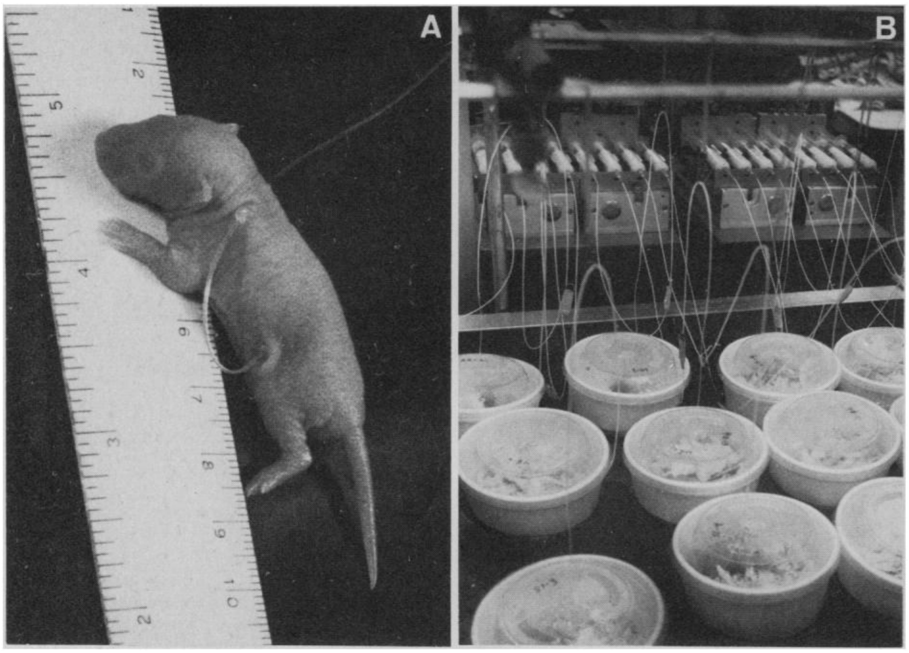

Fig. 1. (A) A 2-day-old rat pup with its i.g. cannula in place. Note the washer securing the tube at the pup's flank and the loop in the cannula before it passes through a fold of skin at the back of the neck. It is secured there by a second washer. (B) Pups are housed in cups which float in a temperature-controlled water bath (the water temperature is maintained at 40°C until the pups are 13 days old and then gradually dropped to room temperature; this temperature program keeps the pups' axillary temperatures at 32° to 36°C). The i.g. cannula (joined to a PE-50 lead) can be seen emerging from the lids of the cups and passing overhead to syringes mounted on infusion pumps. Diet was infused continuously.

about feeding (*6*). Moreover, these techniques are prohibitively tedious and time-consuming (*7*). The technique used here for artificially rearing rat pups, and briefly described below, both eliminates oral feeding experience and, because of its ease of use, has numerous other developmental applications.

The two major components of the technique are a miniaturized intragastric (i.g.) feeding cannula and an incubator housing arrangement. The i.g. cannula is constructed from a length of fine polyethylene tubing (PE-10) with a thin collapsible polyethylene disk attached at its tip (*8*). The implantation procedure is unique in that the cannula is installed via the mouth and esophagus. A pup is lightly anesthetized with ether and placed on its side; then a soft lubricated Silastic tube (*9*) is gently passed through the mouth, down the esophagus, and into the stomach. A length of piano wire (*10*) is inserted into this guide tube and gently pushed down almost to its tip. The wire and tube are located tactually and positioned in the stomach against the left flank, roughly on a line between the two left limbs. Then the wire is pushed out of the tubing, piercing the gastric and peritoneal wall, to emerge through the skin of the left flank approximately 3 mm caudal to the lowest rib. The Silastic tubing is removed, leaving the wire protruding from the pup's mouth and flank, and the end of the cannula without the disk is friction-fitted over the wire at the mouth (*11*). When one pulls the wire protruding from the flank, the cannula is gently drawn through the mouth, esophagus, and stomach to emerge at the flank. As the disk enters the mouth it collapses as it passes down the esophagus and reopens in the stomach to form a tight seal on the inside of the gastric wall. The cannula is secured at the flank with a polyethylene washer (*12*) inserted over the tubing and pressed snug. Another washer is placed 4 cm distal to the first to anchor the cannula which is passed through a fold of skin at the back of the neck. This anchor serves to absorb any stress on the assembly. The entire procedure takes only about 2 minutes and is relatively nontraumatic, requiring no sutures or incisions. A 2-day-old pup bearing an i.g. cannula is pictured in Fig. 1A.

Pups are reared in the warm moist incubator shown in Fig. 1B. They live in individual Styrofoam cups (*13*) which float in a temperature-controlled water bath. Overhead leads carry diet (*14*) from infusion pumps to the pups. The cups insulate the pups from direct contact with the heat source. But, more importantly, because the cup is free to rotate, it turns with the pup's movements; the leads cannot twist, kink, or be torn out by the pup's activity. Maintenance procedures consist of replenishing diet, ensuring that the leads do not clog, and periodically renewing the bedding (*15*).

This artificial rearing technique is successful. Some 80 percent of the rat pups implanted with the cannula between 12 and 48 hours after birth have been successfully reared, in good health, to weaning. Figure 2A compares the growth of pups during artificial rearing to that of normal pups reared in litters of 8 to 14 individuals (data are from five litters). Artifically reared animals grew well (*16*), although their weight at weaning (18 days) was lower than that of normally reared pups [mean of 31.6 g for artificially reared pups as compared with 36.1 g for pups reared normally; $P < .05$ (*17*)]. There was considerable overlap in the body weights (the range for artificially reared pups was 28.7 to 35.4 g; the range for normally reared pups was 29.5 to 41.3 g), and there were no apparent abnormalities in the skeletal growth of the artificially reared pups (for example, the mean femur length in artificially reared pups was 2.8 ± 0.05 cm as compared with 2.8 ± 0.07 cm in pups reared normally). Moreover, artificially reared pups were active and appeared behaviorally competent.

The original question remains: Do artificially reared pups recognize and eat food

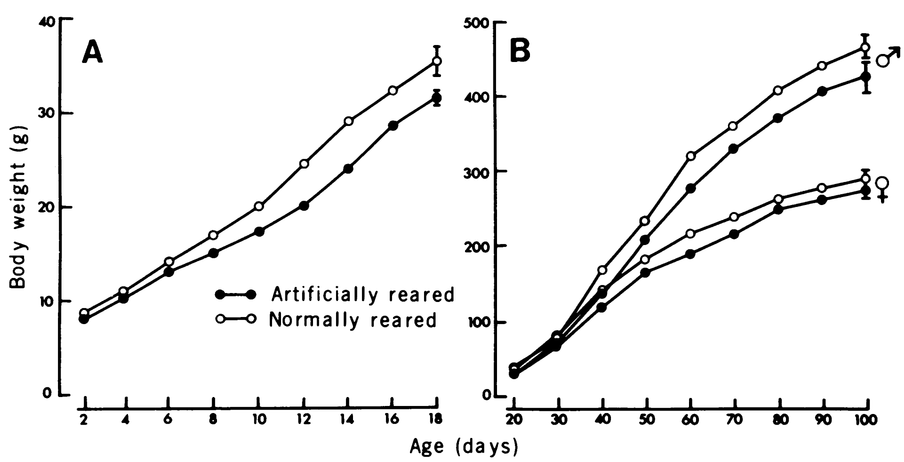

Fig. 2. (A) Mean body weight (± standard error of the mean) of artificially reared (N = 18) animals during the preweaning rearing period compared to the weight of pups reared normally in the litter (N = 18). (B) Postweaning growth, by sex, of artificially reared and normal rats (N = 9 for each curve).

at weaning despite having missed almost the entire suckling experience? And, if weaned successfully, will they grow normally? The following experiment was carried out in an effort to answer these questions.

Eighteen-day-old pups removed from the incubator after artificial rearing or taken from their mothers were put into standard individual cages and deprived of food and water overnight. The next day, artificially reared ($N = 18$) and normal pups ($N = 18$) were presented with either Purina Chow and water, a milk-based liquid diet, or standard formula Noyes pellets (0.045 g) and water. Food and water intake was recorded every hour, and the time that it took each rat to consume 0.5 g of food was determined.

The median times required to eat 0.5 g of food were identical in artificially reared and normal pups eating the liquid and Noyes pellet diets (median, 1 hour; range, 1 to 3 hours in each group), whereas the normal pups ate Purina Chow in 1 hour's time (median) and the artificially reared animals required 2 hours. Observations of the first feedings indicated that the initial bites (or licks) of food occurred early and at similar times in both groups of pups (5 to 20 minutes). Artificially reared rats appeared to walk to the food supply and eat in the same fashion as pups reared normally and seemed to experience no difficulty in identifying or ingesting their first oral food. This behavior occurred despite the fact that the artificially reared animals had no more than 12 to 48 hours of oral feeding experience during the first 18 days postpartum. Every pup offered a dry diet was also observed to drink water within the first test hour. It does not seem likely that the 12 to 48 hours of postnatal experience with the mother could be critical to these findings. One animal that was successfully reared from birth *never* having suckled acted in the same way as the artificially reared pups described above (*18*). Furthermore, the major behavioral transitions in suckling appear later in the nursing period (*2*).

After weaning, artificially reared and normal rats grew at similar rates (Fig. 2B). The small differences in weight ($P < .05$) would probably be expected simply on the basis of the weight differences at weaning (*19*). The well-known sex differences in growth emerged in the artificially reared animals at the same time they appeared in normally reared animals.

In addition to feeding and drinking at weaning, and growing at a fairly normal rate, the artificially reared rats seemed to be as motivated as normal rats to feed or drink. As adults ($N = 6$, each group), they responded in the same way as normal rats to the following situations: food deprivation (4 hours during which liquid diet was available after 24 hours of food deprivation: artificially reared rats consumed 23.3 ± 1.9 ml; normally reared rats consumed 23.0 ± 2.3 ml); water deprivation (4 hours during which water was available after 24 hours of water deprivation: artificially reared rats drank 13.7 ± 0.1 ml; normally reared rats drank 13.3 ± 1.5 ml); and dietary adulteration with 0.02 percent quinine hydrochloride (the 24-hour dietary intake of artificially reared rats was depressed to 81.3 ± 5.7 percent; that of normally reared rats was depressed to 79.7 $\pm$ 6.3 percent). Artificially reared rats also learned to bar press for food and water as quickly as normal rats.

Two new findings emerge from these data. First, participation by the rat in suckling throughout the nursing period is not necessary for food recognition and ingestion at weaning. In fact, even the potentially damaging combination of suckling deprivation, isolated rearing, and maintenance on a synthetic diet had little effect on the general growth and adult ingestive characteristics of the artificially reared rat. The emergence of feeding, therefore, seems highly determined in rats (*20*). The experiential contribution to ingestive behavior is limited. Even though experience with particular maternal diets can temporarily bias diet selection (*1*), experience may effect only subtle changes in ingestive behavior. These influences require closer scrutiny. Second, the normal growth and feeding characteristics of artificially reared rats make them an attractive experimental preparation for developmental studies. Artificial rearing may be particularly useful in behavioral studies where experimental control of early experience is required, in nutritional investigations where the programming of specific diets is desired, and especially in any type of study where an experimental manipulation (for example, neurological, pharmacological, or physiological) would interfere with normal suckling and growth in the litter.

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6. E. B. Thoman, A. Wetzel, S. Levine, *Anim. Behav.* **16**, 54 (1968).
7. A pioneering attempt to make isolate rearing of rats more practical has been reported by M. Messer, E. B. Thoman, A. G. Terasa, and P. R. Dallman [*J. Nutr.* **98**, 404 (1969)]. I present here a development of the i.g. cannula that was described by these authors.
8. The i.g. cannula was made from a 15-cm length of PE-10 tubing (Clay-Adams) (0.28 mm, inside diameter; 0.61 mm, outside diameter). A flange (0.70 to 0.90 mm in diameter) was heat-formed at one end, and the disk (6.5 mm in diameter, cut with a standard paper punch from a polyethylene sandwich bag 0.020 mm thick) was pushed onto the cannula and tight against the flange. The dimensions of the flange were critical. If too large, the flange would damage the esophagus; if too small, it would not retain the disk.
9. Dow Corning Silastic, catalog number 602-101 (0.30 mm, inside diameter; 0.64 mm, outside diameter; length, 6.2 cm). Before being inserted, the tube was lubricated with vegetable oil.
10. A gently curved piece of music wire (0.255 mm in diameter, 8.3 cm long) had a marker bend (about 25°) placed at 6.0 cm from the tip. As the marker reached the mouth of the Silastic guide tube, it indicated that the tip of the wire in the stomach was protected by 0.2 cm of tubing. The wire was also lubricated with vegetable oil, and any difficulty in insertion was overcome by gently shifting the pup's position.
11. The cannula and especially the disk were lubricated with vegetable oil.
12. Washers were made from PE-50 tubing (Clay-Adams) (0.58 mm, inside diameter; 0.97 mm, outside diameter) which fitted tightly over the PE-10 tubing. They were formed by heating the tip of the tubing at a flame, and, after it blistered, pressing it flat against a smooth surface. The washers, which were then cut off the tubing, had a diameter of approximately 2.2 mm.
13. Housing units were Styrofoam dessert dishes (2 mm thick, stock number 8S20, Dart Container Corporation, Mason, Wis.) and their plastic lids; six 0.63-cm holes were punched in each lid. As the animals approached 10 days of age, their body weights became sufficient to capsize their cups. To prevent this, I counterweighted each cup by placing it in another cup which had a long brass bolt projecting from its bottom. Weights were attached to the ends of these bolts.
14. The artificial diet was taken from Messer *et al.* (*7*). Diet was prepared in 400-ml batches and frozen in 10-ml syringes. Syringes were mounted on multiple syringe carriers on infusion pumps (Harvard, model 942), eight syringes per pump. A 2-day-old pup received 2 ml per 24 hours, with the rate increasing thereafter by 0.62 ml per day. Syringes were covered with cold packs (not shown in Fig. 1B) to prevent spoilage of the diet.
15. Two days after cannula installation, the washer at the pup's flank was released and pushed up the tube. This adjustment prevented the cannula from being pulled out by tissue growth and allowed the cannula to move freely within the natural fistula that had formed around it. At times, a suture was taken near the wound and tied around the new position of the washer to keep the pup from pulling the cannula out. It is commonly thought that urination and defecation must be manually stimulated in rat pups, for example, Messer *et al.* (*7*). In the rearing situation reported here, such stimulation was found not to be necessary. It is likely that the movement of the cups in the bath provides sufficient stimulation to produce evacuation of waste, and constant attention to the pup is not necessary.
16. The growth of these artificially reared pups is much better than that usually reported for rat pups hand-fed similar diets [for example, see (*5*)].
17. All variances are expressed as the standard error of the mean; statistical tests were made with a two-tailed *t*-test.
18. The difficulty in artificially rearing pups from immediately after birth may have resulted from the neonatal pup's difficulty in handling the osmotically concentrated diet, or from the pup's failure to get a constituent of the mother's colostrum or its own saliva.
19. E. M. Widdowson and G. C. Kennedy, *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **156**, 96 (1962).
20. This does not appear to be the case in primates [R. E. Miller, W. F. Caul, I. A. Mirsky, *Physiol. Behav.* **7**, 127 (1971)].
21. I thank E. M. Blass for help and encouragement during the course of this work, and F. S. Kraly and S. M. Plaut for suggestions on the manuscript. The research was supported by NSF grant BMS 75-01460 to E. M. Blass and PHS research fellowship MH 58056.
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