Reports

Reproductive Cycle of the California Leaf-Nosed Bat, Macrotus californicus

Abstract. Spermatogenesis is limited to summer and fall, and females are inseminated during the fall concurrent with ovulation and fertilization. While most of the embryonic development is deferred until March, there is slow growth of embryo and placenta during the winter. This sequence is termed "delayed development." Birth is in June, and the young bats are able to join the forage flights by August.

Previous reports of the reproductive cycles and length of gestation in North American bats can be summarized by the description of three general patterns. One type is exemplified by the Vespertillionidae (*1*). This family of bats is characterized by spermatogenesis during the summer only, insemination shortly before or during hibernation, and retention of sperm in the female reproductive tract to fertilize ova released in the spring. The gestation period is 2 to 3 months. In this family *Myotis lucifugus lucifugus* has been the most extensively studied. A second type is illustrated by the Molossidae, but is much less well known. Sherman (*2*) reported that for *Tadarida brasiliensis cynocephala*, a species of Molossidae which does not hibernate, spermatogenesis occurs just prior to and during the period of insemination in the spring. Ovulation is concurrent with insemination, and the gestation period is 11 to 12 weeks. The reproductive cycle of many bats is similar to that of the Molossidae (*3*). A third type is the cycle of *Desmodus*, described by Wimsatt and Trapido (*4*). Members of the family Desmodontidae may be polyestrous, and their gestation period may be quite long.

The California leaf-nosed bat, *Macrotus californicus*, is one of the four species of phyllostomid bats of the southwestern United States. The reproductive cycle is a combination of the three types described above. Study from March 1958 to August 1960 of a resident population of *M. californicus* living in a mine tunnel near Silverbell, Pima County, Arizona, has revealed the essential facts of the reproductive cycle (*5*). The males become reproductively active during July and August. Reproductive activity is evidenced by enlargement of the testes and epididymides that lie near the base of the penis. During July and August the males live apart from the females in small bachelor groups in mines and caves other than the main mine tunnel. In September the males and females recongregate. Insemination, ovulation, and fertilization occur during September, October, and November. The earliest date for fertilization that I have recorded is 20 September, but most females are fertilized during October and all are pregnant by the end of November. By November spermatogenesis ceases and testes and epididymides regress in size, becoming more internal in position.

The next phase of the cycle is most curious. From October until March embryonic development is very slow. This retarded development is not like delayed implantation, since the trophoderm of the blastocyst invades the uterine glands to form an early placental association, and the inner cell mass is more extensive than in cases of delayed implantation. However, the early embryo does increase in size. A series of uteri collected during the winter months showed a progressive expansion of the right uterine horn, but embryonic development is limited to an increase in the number of cells with little differentiation. During March, April, and May fetal development is completed, and birth occurs in June. Elsewhere I have termed this sequence "delayed development" (*5*).

The normal body temperature of active *M. californicus* is 37°C. Although these bats can tolerate reduced body temperatures during the winter, they apparently cannot hibernate, and a sustained body temperature below 26.0° ± 0.5°C is lethal. The lowest rectal temperature that I have recorded is 25.7°C. I suggest that the reduction in body temperature slows the rate of cell division and explains at least part, if not all, of the phenomenon of delayed development. Pearson *et al.* (*6*) have shown that the period of gestation in the lump-nosed bat, *Plecotus townsendi*, is lengthened by reduced body temperature. Nearly all embryonic development of *M. californicus* occurs during the same length of time as in other species of bats; but because of the early retardation, the total gestation period is about 8 months. Only the vampire bat, *Desmodus rotundus murinus*, has a gestation period as long as 8 months (*4*). *Macrotus californicus* might be valuable in the study of early organization since it takes about 5 months for the embryo to reach the primitive streak stage. An unexplained observation is that the ova are released from only the right ovary and placentation occurs, with rare exceptions, in the right horn of the uterus. The reason for the development in the right side is not known.

The young bats are nursed for about 1 month, at which time they get their permanent dentition and join the evening feeding flight. Females born the preceding June participate in the fall mating period, but insemination usually occurs later in October than it does in older females. Although the subadult female is fertilized when she is about 4 months old, most of the embryonic development is delayed until the female is 9 months old. Young males are true subadults during the first fall, and they do not participate in the mating (*7*).

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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

References and Notes

1. W. A. Wimsatt, *J. Mammal.* **26**, 23 (1945).
2. H. B. Sherman, *J. Mammal.* **18**, 176 (1937).
3. E. L. Cockrum, *Trans. Kansas Acad. Sci.* **58**, 48 (1955).
4. W. A. Wimsatt and H. Trapido, *Am. J. Anat.* **91**, 415 (1952).
5. G. VR. Bradshaw, *Mammalia* **25**, 117 (1961).
6. O. P. Pearson *et al.*, *J. Mammal.* **33**, 273 (1952).

7. This paper is a portion of a doctoral dissertation submitted to the Department of Zoology, University of Arizona, in 1961. E. L. Cockrum administered monetary assistance through his National Science Foundation (G-5209) and National Institutes of Health (E-3147) grants.

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Separation of Cell Components in the Zonal Ultracentrifuge

Abstract. Zonal separation of particles in density gradients contained in hollow or tubeless rotors has been explored with the aim of developing a preparative counterpart of the analytical ultracentrifuge. A rotor with a capacity of 1625 milliliters has been tested up to 22,500 revolutions per minute, with sucrose density gradients used. Excellent separation of subcellular particles has been achieved as well as partial separation of the albumin and globulin peaks of serum.

We wish to develop a true preparative counterpart of the analytical untracentrifuge which will achieve zonal-velocity or equilibrium separation in liquid density gradients of particles having different sedimentation rates or densities. In previous work (*1–3*) the amount of sample and the volume of the gradient have been severely limited by the size restrictions inherent in swinging-bucket rotors. Considerable time is required for density gradient production, and care must be exercised in loading and unloading tubes and in acceleration and deceleration. Further, the stepwise nature of most gradient recoveries limits the resolution attainable. Of these difficulties, the most serious is the limitation on sample and gradient size which has made density gradient centrifugation more of an analytical than a preparative procedure.

For the separation of different cell types and of cell particulates, a system is required in which a reproducible gradient of large volume can be quickly produced and in which centrifugation of a sample can be started a few minutes after sample preparation. To separate particles of molecular dimensions, it is necessary to attain speeds in the ultracentrifuge range. We report here the first results with a rotor and ancillary equipment designed to spin gradients of 1200- to 1500-ml volume at speeds up to 40,000 rev/min (92,000g at R_{max}), but used in these studies to only 22,500 rev/min.

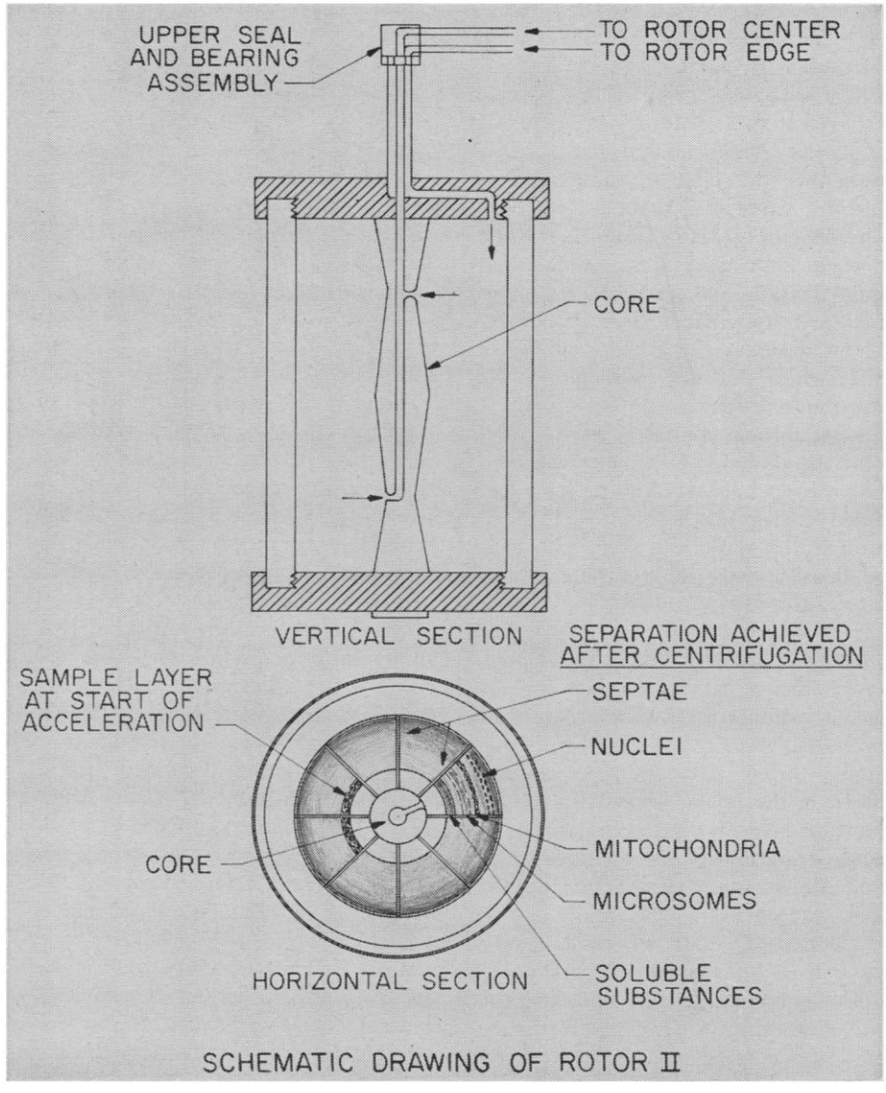

Fig. 1. Schematic drawing of density gradient ultracentrifuge rotor. Gradient is pumped into edge of rotor through upper seal and coaxial tubing. When the light end of the gradient reaches the center of the rotor and begins to flow out through upper seal, the direction of flow is reversed, and the sample gradient is backed into the rotor center. In the horizontal section, division of the rotor interior into sector-shaped compartments is shown. The sample layer is shown in two compartments (left) as it appears immediately after introduction into the rotor. The separation achieved after prolonged centrifugation is also shown (right). The gradient is recovered by pumping 66 percent (wt./wt.) sucrose to the edge of the rotor, displacing the gradient to the center and out through the upper seal assembly.

The basic principles employed in the present zonal ultracentrifuge (ZU-1), which are in many respects similar to those used in the gas ultracentrifuge for isotopic separations, have been developed with the use of four low-speed centrifuge systems (*2*, *4*).

With the background of experience thus gained, we proposed a zonal ultracentrifuge rotor (*5*) and associated systems for high-speed operation with the rotor spinning in a refrigerated, evacuated chamber. The design is shown schematically in Fig. 1. Two rotors have thus far been built. The results recorded here were obtained for rotor II which has a capacity of 1625 ml.

The rotor is hollow and contains an axial core. The core is slotted to hold a number of septa which divide the internal space vertically into compartments having the sector shape required for ideal sedimentation. At the two points along its length where the core is constricted, there are exit ports at the points of greatest constriction. The rotor is supported and driven from be-