# Reproductive Performance in the Microphthalmic Rat.* (31934)

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Female rats with microphthalmia(1,2) have a poor breeding record. A sample of 60 microphthalmic females littered on an average of 51 days after continuous exposure to active males, contrasted with 33 days for 60 normal females similarly handled. Over one-half of the females with bilateral microphthalmia failed to give birth to viable young. In the microphthalmic females giving birth to living young the percentage of litters successfully raised to weaning was about 50% of possible (3). The present report gives observations of the reproductive activities of a small sample of microphthalmic females observed daily for 6 months.

*Methods.* Twice daily vaginal smears and observations of 6 female rats with bilateral microphthalmia were continued consistently for a period of 6 months. These mature females were smeared twice daily for 40 days to determine their estrous cycles, and were then mated with bilaterally microphthalmic males. Pregnant females were isolated about day 18-20 of pregnancy, and their young were weaned when 28 days of age. Females were returned to the breeding cage when a litter was still born or on the day the young were weaned, with no period of rest. Six normal females were treated identically. All animals were housed in the same room, the same food provided, same cage size, etc.

*Results.* The average length of the estrous cycle of the females with bilateral microphthalmia was 5.5 days compared to 4.8 days for the normal colony females. One microphthalmic female, after being placed in the breeding cage with 2 active males, did not mate until her fifth cycle of vaginal cornification. Sperm were found in the vaginal smear of another female on her 9th day of pregnancy. She produced a litter of young 13 days later. Long and Evans(4) report that 2 pregnant rats copulated on day 4 and 14, and another on day 16. One microphthalmic female had 2 long periods of vaginal cornification lasting an average of 7 days.

Significant differences in the reproductive performance between normal and microphthalmic females are given in Table I. Compared to the normal controls—the microphthalmic females have longer gestation periods, a delay in onset of vaginal cornification following delivery, and exhibit difficulty in providing milk for their newborn.

Lisk(5) reports that vaginal plugs are pink or red because of the irritation "of the spines" on the penis of the male rats. Morning and evening vaginal smears revealed that only 11-13% of the copulation smears (sperm present in the smear) contained red blood cells. "Implantation" hemorrhage was present on an average of about day 14 for the microphthalmic females and day 13 for the normal females. Long and Evans(4) reported that this sign in their colony was present from day 14-17. Most of the pregnant microphthalmic females exhibited a "placental hemorrhage" prior to delivery. This sign appeared as early as 4 days prior to delivery, with the most frequent occurrence about 2 days before littering. There was no apparent correlation between this "placental sign" and the presence or absence of stillborn.

Seven of the 18 deliveries by microphthalmic females resulted in no live births. The average litter size at birth was 6.9 for the microphthalmic females and 7.9 for the normal females, with an average of 4.4 live births and 5.6 live births respectively. Of the litters of young born alive to the microphthalmic line about ⅓ of the young starved to death or were eaten early by the female. However, the surviving young from this strain weighed slightly more (5.1 *vs* 4.9) than the young from normal females, probably because of smaller litter size. Normal colony females raised to weaning (28 days) over 80% of the young born alive, compared to about 75% for the microphthalmic females. The smaller sized litters of the microphthalmic females

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TABLE I. Comparison of Reproductive Performance of Microphthalmic with Normal Female Rats.

| | Normal colony | Microphthalmic colony | |
|---|---|---|---|
| No. of females | 6 | 6 | |
| " " pregnancies | 17 | 18 | |
| | Mean ± S.E. | Mean ± S.E. | P |
| Avg length of gestation (days) | 21.8 ± .16 | 22.8 ± .24 | .01 |
| No. of days to first vaginal cornification following delivery, *not* nursing | 10 ± 1.03 | 15.1 ± 1.09 | .01 |
| No. of days to vaginal cornification following delivery; while nursing | 21.4 ± 1.01 | 26.3 ± 2.06 | .02 |
| % of all litters with milk in the stomach on day one | 80 ± 5.89 | 55 ± 3.89 | .01 |

weighed more at weaning than the young from normal females.

Weanlings from females initiating estrous cycles early tended to weigh less at weaning than young from females beginning cycles after day 22. Vaginal cornification had resumed by day 21 (Table I) in most of the nursing normal colony females. Long and Evans(4) reported estrous cycles began about day 25 in their rat colony.

Litter success of the microphthalmic females improved with each pregnancy. About 17% of the litters survived to weaning in the first pregnancy, with 83% of the litters born surviving by the third pregnancy.

The following chronological protocol summarizes the breeding history of 2 females with severe microphthalmia: Female #5003. Bilateral microphthalmia. 108 days of age. 155 g. Average estrous cycle, 4.1 days. Placed in the breeding cage on Feb. 11. Sperm in the Feb. 17 vaginal smear. Diestruum of pseudopregnancy until March 1. Sperm in smear of March 3. "Implantation" hemorrhage March 14-March 18. Isolated March 21. Delivered on March 24: 8 young, 5.0 g, 6 stillborn, 2 alive; no milk in the stomach of either young. All young dead by March 25. Returned to breeding cage March 25.

She showed vaginal cornification March 25th and April 5th, but there were no sperm in the smears. Sperms were present in the morning smear of April 9. "Implantation" hemorrhage April 22-April 26. Isolated on April 29. She delivered on May 2: 9 young, 5.5 g, 2 stillborn, 7 alive. There was no milk in the stomach of any of the young on day one; all young were dead May 4. Returned to the breeding cage May 5. Sperm in the smear of May 13. "Implantation" hemorrhage May 27:30. Isolated June 3. Littered June 3: 10 living young, 5.0 g, milk present in the stomachs of all young. Litter reduced to 7 on June 5. Weaned 7 young on July 3, 43 g. The young females from this litter had vaginal opening at the average age of 57 days.

Female #5018. Bilateral microphthalmia. 173 g. Average estrous cycle 4.6 days. Placed in breeding cage Feb. 11. Vaginal cornification on Feb. 12 and Feb. 18 with no copulation. Sperm found in the smear of Feb. 23rd. "Implantation" hemorrhage March 7-10. "Placental" hemorrhage beginning March 17th. Delivered March 19: 5 young, 2 stillborn, 3 alive, no milk in the stomachs on day 1. All young dead on March 20. Blood of "resorption" in the vaginal smears of March 26-29. No biopsy performed. Sperm in the smear of April 1. "Implantation" hemorrhage April 11-April 19. Delivery on April 23: 4 stillborn, 1 alive, 5.6 g. Milk present in the stomach of the one living on day one. However, all young dead on April 24. Returned to breeding cage April 24.

Sperm found in the morning vaginal smear of May 5. "Implantation" hemorrhage, May 16-May 20. "Placental" hemorrhage began on May 27. Delivery on May 28: only 1 dead young found. Blood of "resorption" from May 31-June 4. No biopsy performed. Sperm in the smear of June 16. "Implantation" hemorrhage from June 23-July 1. "Placental" hemorrhage began July 5. Fetuses palpated on July 11. Hemorrhage of delivery on July 12. No young found, no fetuses could be

palpated. Presume that the stillborn were eaten.

*Summary.* Female rats with bilateral microphthalmia are frequently not mated by microphthalmic males during their initial estrous cycles in the breeding cage. Several cycles may occur in the presence of males with no mating. Gestation in microphthalmic females is longer than in the normal colony females, and the litter size tends to be smaller. A higher proportion of stillborn young are born to microphthalmic females, and of the young born alive fewer survive to weaning age than is true of the young born to normal colony females.

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## SV-40 Virus Growth and Cytopathogenicity in a Serial Rabbit Kidney Cell Line.** (31935)

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Simian vacuolating virus SV-40 is known to grow in primary and serial cell lines of African green monkey *(Cercopithecus aethiops)* origin(1) eliciting a characteristic vacuolating cytopathic effect (CPE). Although SV-40 virus has been shown to multiply in a variety of cell lines from other sources, it did not produce cytopathic effect useable for virus assay. However, upon protracted incubation with SV-40 virus, all the cell lines examined exhibited changes in cell morphology and growth patterns(2,3,4).

The observation by Black and Rowe(4) that primary rabbit kidney cells could thus be transformed led us to examine a serial rabbit kidney line, namely MA-111, for its response to SV-40 virus inoculation. SV-40 virus produced a fulminant cytopathic effect in MA-111 cells. This report describes some of our observations and indicates the usefulness of this cell line for the titration of SV-40 virus and for the production of SV-40 viral and CF antigen in cells from a non-simian source.

*Materials and methods. Tissue culture.* Primary African green monkey kidney cells, BSC-1(5) cells of low (34-65) and high (137) passage levels, and primary rabbit kidney cells, as well as a developmental cell line, MA-134 (African green monkey kidney), were obtained from Microbiological Associates, Inc.

The MA-111 cell line, also obtained from Microbiological Associates, was developed by Monroe M. Vincent* from newborn rabbit kidney cells. They are now in their 104th passage. Karyotype analysis kindly carried out by Dr. P. Price* showed that "MA-111 was heteroploid, with a small percentage of cells having 44 chromosomes all of which fell into the normal rabbit grouping." Similar observations were made by E. M. Earley† (personal communication). All cells were maintained at 34°C on Eagle's minimum essential medium(6) containing 3% agamma calf serum,‡ 100 units of penicillin/ml, 100 μg of streptomycin/ml, and 4 mM glutamine. Medium changes were done every 4 days.

*Virus.* SV-40 virus strain #776 (originally from Dr. H. M. Meyer) was obtained from the Laboratory of Infectious Diseases.§ It was propagated on African green monkey kidney

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