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Manuscript received July 2, 1952.

Unusual Reproductive Phenomena in Rodents

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It was observed while breeding white rats and mice for experimental purposes that occasionally a female would deliver two successive litters without having been bred a second time. It has been the practice of the author to segregate pregnant animals in brood cages from the time they were obviously pregnant until the young were of weaning age. Over a period of years, three unequivocal cases of this phenomenon have been personally observed. One recent example of anomalous pregnancy in a guinea pig is also included.

Case I: White rat Y, female, was caged with two males. On Sept. 18, 1940, female was isolated because of obvious pregnancy. On Sept. 20, 8 healthy young were born, all of whom survived. On Oct. 15 this same rat gave birth to another litter of 11 young. The first litter was between 3 and 4 weeks old at this time and was removed from the cage. Of the second litter, 5 were dead at birth, 4 died before weaning age, and 2 lived. Twenty-five days intervened between the successive litters. This female rat had been separated from all other rats two days before the first litter was born, and from all but her own young for 27 days before the birth of the second litter. The remaining history of this animal was uneventful, although she survived to July 15, 1941, when she was sacrificed.

Case II: The author has personal knowledge of this animal, and it is presented on personal communication from A. Barber (*1*). Mouse L-7, *dba* female, and her own young, L-16 (male) and L-15 (female), were accidentally allowed to remain together until the young were mature and mating occurred. On July 29, 1950, each animal was isolated, and on Aug. 8, L-7 delivered 6 normal young. On Sept. 1, 24 days later, the mother was isolated, and on Sept. 3 she delivered 7 normal young. This mouse had not been mated since July 29, or 36 days before the birth of the second litter. The mice in both litters were *dba,* and of mixed sex.

Case III: Deer mouse (*Peromyscus maniculatus gambelii*), brown, adult, female was isolated Dec. 18, 1950, because of pregnancy. On Dec. 27, 2 young, about 1 week old, were found when the cage was changed. On Jan. 15, 1951, 4 newborn young were found when the cage was changed on this date. Four days later the older mice, 1 male and 1 female, were removed. The second litter had velvety coats, but one was a runt that subsequently died. The survivors of this litter were 2 females and 1 male. The time between isolation of the mother and birth of the second litter was 28 days, and the time between the birth of the first and second litters was 24–26 days. The *Peromyscus* female was observed for 9 months after this, but no unusual behavior was noted. Observation of these animals is more difficult than with other mice because of their shyness and their burrowing habits.

Guinea pig: Albino female, bred to an albino male, had previously had two pregnancies. The week of Apr. 14, 1952, this animal was isolated because of pregnancy, and during the week of May 12 she delivered 4 healthy young. She was placed with two other sows and their young (3–4 weeks old) when her litter was about 2 weeks old, because of a housing emergency. She was left in this cage for less than 24 hr. None of the other pigs were albinos, but on June 27 this animal gave birth to 3 healthy, well-developed, albino female young. The time between the birth of these two litters was 42–46 days, and the interval between the time she was isolated and the birth of the second litter was 70–74 days.

Two phenomena have been reported to occur in mammals, including rodents, which involve irregularities in pregnancy. One of these, delayed implantation of the blastocyst stage, was reported and reviewed by Hamlett (*2*). This occurs normally in certain animals, and irregularly in others, including rats and mice, according to him. However, if delayed implantation is the explanation of the three cases (one rat and two mice) reported here, the blastocysts must have survived during some portion of the first pregnancy, been retained in the uterus during delivery, and been implanted following delivery of the first litter.

The second phenomenon, superfetation, has been reviewed by Rollhäuser (*3*), who reported a case in a mouse. The essential indication that this has occurred is the delivery of a second litter at a shorter interval after the first than the normal gestation period. Rollhäuser found reports of time intervals from 7 to 16 days between the birth of the two litters in rats and mice. It seems unlikely that this is the explanation of our rat and mice cases, as the time intervals between litters in these animals was longer than the normal gestation period. The case of the guinea pig may well be an example of superfetation, however, since the time interval between the delivery of the two litters was much shorter than the minimum gestation period for guinea pigs, according to Ibsen (*4*). Another argument in favor of superfetation in this case is that the time interval between the isolation of this animal and the birth of the second litter (70–74 days) coincides with the maximum gestation period reported by Ibsen.

It is possible that a combination of superfetation late in pregnancy, combined with delayed implantation of the blastocysts, may account for the observed results. However, the case of the *dba* mouse is difficult to explain on this basis, inasmuch as 10–15 days must have intervened between the last possible mating and the implantation of the blastocysts. Parthenogenesis is improbable because at least two of the last litters were made up of male and female animals.

This phenomenon may be more common than the literature would indicate. Two people who breed animals for experimental work have told the author that they have observed it occasionally, but did not have adequate records on individual animals. The only reference the author has been able to find of such cases is the one by Burrows (*5*), who reports ten examples in Wistar rats and two in *dba* mice. Five of these animals had records of the date of isolation, and in these cases the intervals between isolation and the birth of the second litter were 24–29 days. Burrows quotes two references to this phenomenon which he found in the literature, involving two cases in mice and one case in a rabbit. This anomalous type of pregnancy is not confined to any one genus, having been observed in the cases reported here, in *Rattus, Mus,* and *Peromyscus* (*6*). It is not confined to highly inbred strains, as the *Peromyscus* mouse was one of a stock carrying both white coat color and hairlessness, apparently as recessive characteristics.

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Manuscript received July 25, 1952.

The *in Vivo* Conversion of Glycine to Serine

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The conversion of glycine to serine by the liver has been shown to occur *in vitro.* A similar reaction has not been previously demonstrated in the liver of the intact animal. It will be shown that under varying conditions the metabolic path of glycine in the liver of the intact mouse is similar to the *in vitro* reaction.

Nine "A" strain male mice were equally divided into three groups. Group I was supplied with its normal diet, Group II was given only a saturated solution of glucose in water, and Group III was allowed only water. This diet was continued for 72 hr.

Each mouse was then injected with 1.728 mg of glycine-2-C^{14} (8.6 μC) of C^{14}-methyl-labeled glycine, and one mouse from each group killed with ether 2, 4, and 6 hr after the administration of the radioglycine.

TABLE 1

Group	Time (hr)	Amino acids	Relative radioactivity
I (Normal diet)	2	Glycine	++++
	4	"	++++
		Serine	++++
	6	Glycine	++++
		Serine	++++
II (Glucose + water)	2	Glycine	++++
		Serine	++
	4	Glycine	++++
		Serine	+++
	6	Glycine	++
		Serine	++
III (Water alone)	2	Glycine	++++
	4	"	+++
		Cystine	+
	6	Glycine	+++
		Cystine	+
		Serine	+++

The liver was removed *in toto* from each animal and hydrolyzed in 6 *N* HCl over a steam bath for 18 hr. The hydrolysate was filtered, and salts were removed by ion exchange technique. The solution was concentrated and chromatographed two-dimensionally on No. 1 Whatman filter paper (*1, 2*), using phenol and butanol propionic acid. After the papers had dried, they were radioautographed on Eastman No-Screen x-ray films. The amino acids on the chromatograms were then developed with a spray of 0.1% alcoholic ninhydrin. By superimposing the respective radioautographs on the developed chromatograms the radioactive amino acids could be determined. This activity must have been derived from the injected radioglycine. A small fragment of each liver was also examined microscopically to determine any cytological changes that were due to diet.

Table 1 summarizes the results. Two hr after injection, only the glucose-fed animal produced a moderate amount of radioactive serine. After 4 hr the glucose-fed animal had produced a little more radioserine, and in the normal-fed animal radioglycine and radioserine were present in approximately equal concentrations. Only after 6 hr did the water-fed animals convert glycine to serine. In addition they showed the presence of a small amount of radiocystine not present in either of the two other groups.

Liver biopsy revealed no abnormality in the normal group, minimal vacuolization was present in the glucose-fed animals, and marked degenerative changes were seen in the water-fed group.

Winnick et al. (*3*) showed that after incubation of liver homogenate with C^{14}-labeled glycine, 60% of the isotopic carbon was found in serine. Sakami (*4*) felt that such conversion occurred by means of the combination of glycine with formate and provided

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