12. ———, Ann. Int. Med., 1967, v66, 1042.
13. Prockop, D. J., Davidson, W. D., New Eng. Med., 1964, v270, 269.

Received July 21, 1967. P.S.E.B.M., 1967, v126.

The Reproductive Performance of the Laboratory Mouse: Maternal Age, Litter Size and Sex Ratios.* (32541)

ROBERTS RUGH AND MARLIS WOHLFROMM
Radiological Research Laboratory, Dept. of Radiology, Columbia University, New York City

The description of the normal reproductive activity of mice cannot be generalized because it varies from strain to strain(1,2). Age and previous breeding experience are also contributing variables. Nevertheless, there is merit in presenting statistical data for a particular strain of mouse used frequently in research laboratories. The strain here described is known as the CF1 from Carworth Farms, but the data would probably be quite similar for other strains.

Experimental procedure. Virgin females of 3 months of age were earmarked for individual identification and 350 were selected for continuous matings (except for 3-5 days after delivery) with adult males of the same strain. The offspring were removed at birth, analyzed and discarded so that the female could soon become pregnant again. Data were collected through the first 4 pregnancies and since no nursing was allowed, the time-lapse for the 4 pregnancies was reduced.

At birth the members of each litter were counted, including those born dead, those that had persistent amnions, or showed anomalies of the central nervous system, or were eaten by the mother. Subtracting these delivered but abnormal mice from the total, the litter size of "normal" mice was established for each mouse and for each of the 4 pregnancies. In addition, the sex of each mouse was determined at birth so that the sex ratio of successive litters could be evaluated. Thus, the data herein presented come from 4 successive pregnancies of 350 selected female CF1 mice.

* This investigation was supported by Contract AT-(30-1)-2740 for the U. S. Atomic Energy Commission and USPHS Research Grant RH-81, National Center for Radiological Health.

In addition 557 litters from 4 different groups of females were analyzed. The females were young and mature virgins, multipara females and ex-breeders of 10-12 months of age. Since these pregnant females were dissected at 18 days the data include resorptions, anomalies, and information on total implantations.

Experimental data. Aside from abnormal or destroyed newborn mice, some 13,508 "normal" newborn mice were available for this study. In other studies it has been established that the average implantation number for this strain of mouse is close to 11. When the average litter size is determined for all of the 350 mice it is shown that the first litter had an average of 8.40 and the 4th litter an average of 10.28, with increments for the 2nd and 3rd litter over the 1st. The 5th litter of 128 of these mice averaged 10.35 offspring. Subsequent litters tend to decrease in number.

If one lists those pregnancies which result in litters of different sizes, it becomes apparent that among the primipara mice the largest group have litters numbering 9, while in the 2nd, 3rd, and 4th litters, the greatest number of mice produced 11 offspring. Thus, not only is the average litter size increased with reproductive activity (or successive litters), but all litters after the 1st have their greatest size frequency at 11 offspring. Individual records vary greatly, however, with some primipara mice having a low litter size of 1 and others with a high of 14, while mice having their 4th litters varied from a low of 1 to a high of 18. The data indicate that the 1st litter of these mice is, on the average, the smallest.

The total percentage of anomalous mice at birth (dead, with amnion, with gross anomalies, or eaten by the mother for whatever reason) increases from 2.29% to 3.26% with age and reproductive experience of the mother. Data on implantation number or pre-implantation death are not included in this study.

Mice were allowed to mate as soon as the female was in estrous after the previous litter and the data indicate that even in groups of 25 pregnancies and 260 ± offspring, the sex ratio data varied considerably. Nevertheless, it always favored the males with an overall percentage of 52.8% males and 47.2% females among the 13,508 mice. The range of variation of male/female offspring was from 51.2/48.8 to 54.5/45.5. Now that we limit timed matings to a brief exposure of 45 minutes to 2 hours a study is in progress to determine whether time of day (hence phase of ovulation) may be a factor in determining the sex rate.

In a separate study the reproductive activity of young and older virgins was compared with the reproductive production of older but multipara females and of females known as ex-breeders, which had been used up to about 10 months of age by the supply house to produce mice.

A total of 557 litters were produced by these 4 groups of females with litters averaging from 7.63 for the ex-breeders to 10.35 for the older but multipara females. The average size of litters for both young and older virgins was about the same, and better than for the ex-breeders which were apparently exhausted physiologically.

These mice of varying ages and reproductive history were sacrificed at the termination of their pregnancy and it was possible to determine the implantation numbers, as well as to list all of the anaomalies that survived to term before any were destroyed by the mother (Table II).

TABLE II. Age, Prior Breeding History, and Reproduction in CF1 Mice.

	Virgins, 3-5 mo	Virgins, 7-9 mo	Multi-paras, 7-9 mo	Ex-breeders, 10-12 mo
No. of litters	164	168	128	97
Avg implantations	11.42	11.04	12.36	10.37
Avg litter size	9.48	9.01	10.35	7.63
Normal offspring	83.03%	81.57%	83.70%	73.65%
Stunted offspring	1.97%	3.17%	2.52%	6.36%
Dead fetuses	1.31%	1.02%	1.07%	1.88%
Resorbed *in utero*	13.18%	13.90%	12.57%	17.89%
Anomalous	0.48%	0.32%	0.12%	0.19%
Males	53.80%	52.60%	50.90%	51.70%
Females	46.20%	47.40%	49.10%	48.30%

Litter size for the young virgins ranged from 1 to 16 (1 to 14 for the ex-breeders), but it was interesting to note that the preponderant litter size for all groups was between 9 and 11. This preponderance was not great for the ex-breeders (17.52%), but was considerable for the older multipara females (23.43%). Also, it was noted that the multipara females never had small litters such as 1 to 4, as did most of the other categories.

Summary. When a large group of CF1 female mice are followed individually for their reproductive performance, it becomes

TABLE I. Litter Chronology and Size in CF1 Mouse.

	First litter	Second litter	Third litter	Fourth litter
Total litters	350	350	350	350
Total offspring	2940	3417	3560	3591
Average litter size	8.40	9.76	10.17	10.28
Total males	1601	1748	1878	1903
Total females	1339	1669	1682	1688
Ratio: males/females	54.5/45.5	51.2/48.8	52.8/47.2	53.0/47.0
Dead	1.33%	0.71%	0.93%	0.73%
Eaten by mother	0.47	0.48	0.63	0.70
Persistent amnion	0.33	1.40	1.41	1.51
CNS anomalies	0.10	0.08	0.08	0.32
Other anomalies	0.06		0.05	
Total not normal	2.29%	2.67%	3.10%	3.26%

evident that the first litter size is the smallest and the size gets progressively larger at least to the 4th litter. Among the 13,508 normal offspring examined, following continuous mating procedures, 52.8% were males and 47.2% were females. The number of abnormal mice found in otherwise untreated females of the CF1 strain is never high (2.29% to 3.26%), but it does increase with successive litters from the 1st to the 4th. Among the other ("not normal") mice there were dead and/or stunted fetuses, some were eaten at birth by the mother for reasons unknown, some had persistent amnions, and others had anomalies, primarily of the central nervous system. Virgin mice becoming pregnant for the 1st time, whether young (3-5) months or older (7-9 months) do not have as many implantation sites as do the multipara females of 7-9 months. The average litter size is greatest among the multipara mice, more so than among the ex-breeders or virgins of any age. While males are always produced in greater numbers than females, in all 4 categories, for some reason the multiparas approach closest to a 50/50 ratio. It must be pointed out, however, that these data involve only 128 litters so that the data may not be as reliable as that presented above, from a vastly larger group of mice.

1. Green, E. L., "Biology of the Laboratory Mouse" McGraw-Hill Book Co., New York, 1966.

2. Rugh, R., 1967 "Reproduction and Development of the Mouse" Burgess, Minneapolis, in press.

Received September 27, 1967. P.S.E.B.M., 1967, v126.

Effect of Phytohemagglutinin on Skin Allograft Survival in Mice.* (32542)

RONALD L. ST. PIERRE† JOHN B. YOUNGER,‡ AND CHESTER M. ZMIJEWSKI
(Introduced by D. T. Smith)
Division of Immunology, Duke University Medical Center, Durham, N. C.

Phytohemagglutinin (PHA) has been the subject of considerable interest since the discovery of its activity on lymphoid cells *in vitro*(1). Despite the vast amount of literature pertaining to the "blastogenic" effect of PHA, the nature of this phenomenon is obscure. The *in vivo* effects of PHA are even less well understood than the *in vitro* response. *In vivo* studies have been undertaken in man(2,3), rodents(4-10), and dogs(11).

The "blast-like" lymphoid cell evoked by PHA *in vitro* strongly resembles morphologically the changes taking place during the cellular response to antigenic stimulation. PHA could thus be interpreted as having an enhancing effect upon the immune capacity of lymphoid cells. The results of *in vivo* studies are, however, inconclusive, *e.g.*, it has been found that PHA enhances antibody production(10) and that PHA suppresses antibody production(4,9). The present study was undertaken to examine, *in vivo*, yet another phase of the immune response, that of allograft rejection.

Materials and methods. Animals. Adult male C57Bl/6J and DBA/2 mice, 40-60 days of age were used throughout this study.

Phytohemagglutinin. Phytohemagglutinin—P (Difco Laboratories, Detroit, Mich.) was rehydrated with sterile 0.85% saline prior to i.p. injection.

Skin grafts. Untreated donor mice of both strains were sacrificed and the abdominal hair removed with clippers. After a zephiran scrub, full-thickness pinch grafts of approximately 10 mm were taken and placed in sterile 0.85% saline until used. Recipient mice were anesthetized by 0.1 cc (0.6 mg) i.p. injections of diluted Sodium Pentobarbital (Barber Veterinary Supply Co., Inc., Fayetteville, N. C.). Hair was removed from the back with

* Supported in part by Nat. Inst. Health Grant HE 08421.

† On leave of absence from Dept. of Anatomy, Ohio State Univ. College of Med., Columbus, Ohio.

‡ Recipient of Special Fellowship from Nat. Inst. Health 1-F3-HD-35, 941-01.