free access to laboratory chow. The rotating wheels, 6¾ inches in diameter, were lined with a plastic film in which fine carborundum powder is embedded for traction. Rotation is recorded remotely by electric counters activated by microswitches.

After approximately 3 weeks in the wheel cages, the mice stabilized their activity. The cages were then placed in the chambers, one in smog, the other in purified air. At intervals of 24 hours, the cages were exchanged between chambers, the smogged mouse being placed in filtered air, the filtered air mouse in smog. This process was repeated for a total of 6 days in light smog and 6 days in heavy smog; there was a 1-week interval in the filtered air chamber between the two periods of exposure to smog.

Figure 1 is a semilogarithmic plot of the daily activity records of two individual mice throughout one experiment. The smog concentrations in parts per million (ppm) for each exposure day are shown at the bottom. The regular manner in which low concentrations of smog diminish the wheel-turning is obvious and significant ($P = <.001$), by analysis of variance, as is the greater inhibition which occurred after the smog concentration was increased. The ozone concentration in the first series of exposures corresponds to a first-stage alert in Los Angeles (0.5 ppm), although the total oxidant values are somewhat higher. These experiments are easily repeatable with different kinds of wheels. Thus far, we have shown reduced activity in smog with a total of 14 mice. Furthermore, a decrease in activity is noted for at least 3 weeks when the mice remain in the smog chamber. The activity techniques, though little used for the study of disease, may be sensitive indicators of subclinical disturbances (*14*).

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Preservation of Honey Bee Semen

Abstract. Fertilized eggs have been obtained from queen honey bees (*Apis mellifera* L.) inseminated with sperm that had been stored in vitro at above-freezing temperatures for up to 68 days. The effects of various experimental storage treatments on semen are described. Semen shipped by ordinary mail has been successfully used for artificial insemination.

In some species of insects the semen is stored in the spermatheca of the female after mating. Sperms remain alive in the spermatheca from a few days in certain flies to a few years in some ants and in the honey bee (*Apis mellifera* L.). Queen ants are reported to have laid fertile eggs 15 years and queen honey bees 7 years after mating (*1*). Artificially inseminated queen honey bees have been known to lay fertile eggs for 3 years.

The spermatheca of queen bees is spherical and covered with a network of tracheae. Dissection of queens a few hours after death shows disintegration of all digestive and reproductive organs except the spermatheca. This organ appears fresh, and the enclosed sperms continue to be motile and have been used to inseminate other queens, an indication that the organ has a relatively impermeable membrane.

Attempts at low temperature storage of bee semen at Baton Rouge, La., have been unsuccessful. Therefore, experiments were undertaken to develop some other method of preservation.

Semen was collected from the ejaculate of 5 to 25 drones (*2*) and placed in capillary tubes 1.8 to 2.0 mm in diameter. Pooled samples that had been thoroughly mixed would have been desirable to eliminate differences in the drones' fertilization capacity, but the mixing of sperms in pooled ejaculates is not possible with present techniques (*3*).

Queens were inseminated by the method described by Mackensen and Roberts (*2*). Virgin queens were anesthetized, and semen was placed in their oviducts with a syringe. With this method, several million sperms usually reach the spermatheca and less than 5 percent of the queens fail to survive insemination. The inseminated queens were kept in small colonies of only a few thousand bees, so that the rate of egg laying was no more than 300 to 400 per day.

The first experiment was designed to determine the effect of the following environmental conditions on the viability of sperm stored from 7 to 33 days; (i) dilution with different media, (ii) replacement of the air atmosphere with various gases, and (iii) temperature. The diluent materials included a Ringer-buffer mixture, Ringer-buffer-fructose mixture (*4*), bee blood, and royal jelly. The volume of diluent was not more than the total volume of semen. After the diluent and semen were mixed in some of the tubes, the air above was replaced with carbon dioxide, nitrogen, or helium, by injection from a finely drawn glass tube; the tubes of semen were sealed by heating immediately after removal of the gas jet.

Of 105 queens inseminated, 31 produced fertile eggs and 17 others had sperm in the spermatheca but either did not lay fertile eggs or laid so few that their numbers were considered unreliable. Table 1 shows the storage treatments of sperm used with 14 queens that produced fertile workers. All queens that received semen treated with carbon dioxide died. Semen diluted with royal jelly or bee blood coagulated and could not be transferred to the inseminating syringe. Many of the tubes diluted with Ringer-buffer and Ringer-buffer-fructose had partially coagulated semen. Some tubes stored for 2 weeks or longer showed contaminating microorganisms, and the semen in them was

Table 1. Method of storage of honey bee sperm at defined temperatures.

Days in storage	Temp. (°F)	Gas in storage tube	No. of queens fertilized
Semen in Ringer-buffer solution adjusted to pH 7.4			
33	Room	Nitrogen	2
21	Room	Air	1
Undiluted semen			
29	35	Air	2
22	Room	Air	2
16	Room	Helium	2
		Nitrogen	2
15	90	Nitrogen	2
	35	Air	1

Table 2. Effect of length and temperature of storage of honey bee semen on success of queen insemination.

Days in storage	No. of queens that laid		No. of queens that died
	Fertile eggs	Infertile eggs	
Stored at room temperature			
68*	1	1	2
44	6	1	4
43	2	1	
31*	3		
29	1		3
Stored at 35°F			
43		2	5
31*		4	
29			5
Stored at 90°F			
68*		1	1
31*	1		2
27	3	4	2

* Chlortetracycline added.

discolored or more viscous than normal.

In the second experiment the effect of temperature on the fertilizing capacity of the sperm was studied by storing the sperm in sealed tubes at room temperature and 35° and 90°F from 27 to 68 days. In this experiment 55 queens were inseminated. Thirteen that had been inseminated with semen stored at room temperatures (72° to 86°F) and four inseminated with semen stored at 90°F produced fertile eggs (Table 2). No fertile eggs were obtained from queens inseminated with semen stored at 35°F. Storage was considered successful if at least half the eggs of each queen were fertilized. Sperms stored 4 weeks or longer at room temperatures or above remained viable. Microscopic examination showed that a number of dead queens had living sperms in the spermatheca. The use of better techniques for sterilization and an antibiotic, chlortetracycline, reduced contamination by microorganisms.

Four tubes each containing semen collected from about 20 drones were sent by regular mail to Madison, Wis. Of ten queens inseminated by O. Mackensen with this semen, one died, eight laid all fertile eggs, and one laid both infertile and fertile eggs. Counts of sperm cells in the spermathecae of the nine live queens compared favorably with counts in queens that had received an equivalent amount of fresh semen.

Why these sperms remained viable for such long periods is not known. An explanation of their longevity may be applicable to storage of sperm of other animals. Studies of the artificial preservation of sperm of domestic animals, especially the bull, have received considerable attention since the discovery that glycerol acts as a protective agent (*5*), permitting successful storage at low temperatures. Most of the work on mammalian sperm preservation has been done at very low temperatures, and successful inseminations have been made with sperm frozen 3 years (*6*). Sperms have remained alive for relatively long periods in the ligated epididymis of a number of mammals (*7*), and recently some success has been obtained in the preservation of bull semen at above-freezing temperatures when a continuous flow of physiological fluid passed dialyzing tubes containing the semen (*8*).

Mann (*9*) has shown the importance of fructose in the metabolism of semen. We have found fructose to be present in fresh bee semen, but it is rapidly metabolized, and 40 minutes after ejaculation it has disappeared. Obviously, a supply of fructose is not necessary to survival of honey bee sperm, and it is surprising that sperms can survive under so many different environmental conditions. It may be that sperms stored in the spermatheca receive little or no nourishment and have an extremely low metabolic rate. If so, it should be possible to develop a successful method for storing sperms for long periods of time.

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Effects of Deprivation and Scheduling on Water Intake in the White Rat

Abstract. Two studies are presented to demonstrate that the consummatory behavior of drinking in the rat is under the control of duration of water deprivation and that intake after deprivation is related to variation in the scheduling of the animals' opportunities for drinking.

Miller (*1*) has recently pointed out that different measures of what is held to be a unitary process—for example, the drive state of thirst—on occasion show rather wide variation one from another. The present report (*2*) advances evidence that such variation occurs not only when different classes of behavior are observed as operational measures of the same drive, but also under circumstances where a single indicator varies with changes in laboratory routines.

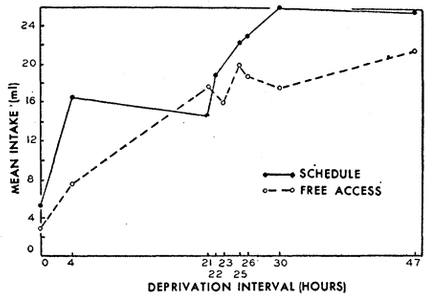

Fig. 1. Amount of water drunk during a test period of 1 hour following water deprivation of varying intervals for animals with free access to water and for animals with access to water limited to 1 hour a day.

In the first study, five albino rats of the Wistar strain were kept in individual cages in a darkened room over a period of several months. For the first 5 weeks of the study, the animals were permitted access to water for 1 hour a day (23-hour deprivation schedule); at the end of this period a series of deprivation tests was begun which extended over a period of 8 weeks (schedule condition). Two observations of drinking during a test period of 1 hour were made after deprivation of 4, 21, 22, 25, 26, and 30 hours, and one observation was made after 0 and one after 47 hours of deprivation. During this time, food was freely available to the animals, and, on days when a test was not scheduled, the animals drank at the usual 23-hour deprivation interval.

When these observations were completed, the animals were returned to free access to water as well as to food for 3 weeks, after which drinking tests were run at the same deprivation intervals used in the schedule condition. In this case, however, the animals were deprived of water *only* during the test interval (free access condition).

The results of the study are shown in Fig. 1. For animals on a free access base line, there is a relatively regular relation between deprivation interval and amount drunk. Intake in this condition shows a statistically significant increase between 0 and 4 hours of deprivation ($p < .05$), and between 4 hours of deprivation and all other intervals ($p < .01$), but there is no significant variation among deprivation intervals of 21, 23, 25, 26, and 30 hours. On the other hand, the amount drunk from a schedule base line shows a striking inflection near the deprivation interval associated with scheduled drinking. This inflection is sharp enough to produce statistically significant differ-