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.

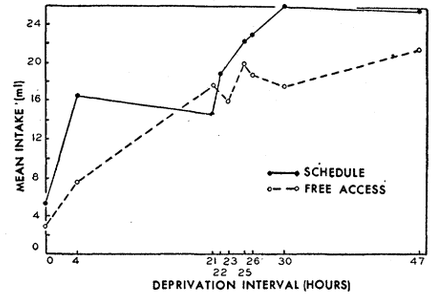

Fig. 1. Amount of water drunk during a test period of 1 hour following water deprivation of varying intervals for animals with free access to water and for animals with access to water limited to 1 hour a day.

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-

ences in intake between 21- and 22-hour deprivation, between 22- and 25-hour deprivation, and between 25- and 30-hour deprivation (all differences at $p < .01$). It is interesting, too, to note that, although a significant increase in intake occurs between 0- and 4-hour deprivation in the schedule condition, there is a nonsignificant *drop* in intake between 4- and 21-hour deprivation.

Scheduling of drinking on a once-a-day cycle seems to have the effect of reducing differences in intake between some relatively short period of deprivation (in this case, 4 hours) and longer periods up to the deprivation interval associated with scheduled drinking, and then of producing a sharp rise in intake at deprivation intervals longer than the one normally associated with scheduled drinking.

A second study was run in order to make a closer examination of intake at the lower end of the deprivation range. Ten albino Wistars were studied, five with free access to water at all times other than test intervals and five under 23-hour scheduled deprivation. Tests of water intake were run at 0, 1, 2½, 4, 5, 6, and 12 hours of water deprivation. In this study, the results of which are shown in Fig. 2, intake over the first 10 minutes of the test hour was analyzed because it had been shown that intake during the shorter period adequately represented intake over the entire hour.

It should be noted in Fig. 2 that, as the first study suggested, the rise of intake with deprivation appears to be sharper in the schedule group than in the free access group. For example, intake for the schedule group was significantly different ($p < .01$) between the lower deprivation intervals (1- and 2½-hour) and the higher intervals (6- and 12-hour). The rise of intake in the free access group, although regular, did not reach conventional levels of statistical significance.

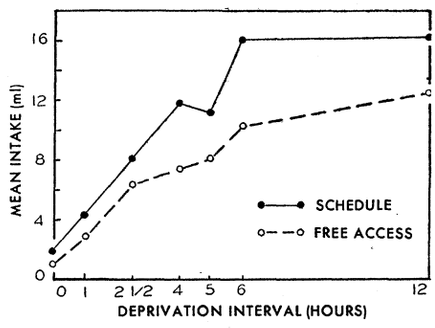

Fig. 2. Amount of water drunk during a test period of 10 minutes following water deprivation of varying intervals for animals with free access to water and for animals with access to water limited to 1 hour a day.

These studies confirm the observation of earlier workers (*3*) that a sharp rise in consummatory behavior takes place between 0 and 4 to 6 hours of deprivation, but they also suggest that part of this effect may be ascribable to the influence of caretaking schedules. Animals which have been "trained" to drink every 24 hours will show distortions of intake when compared with animals tested from a base line of free access to water. These distortions suggest caution in the use of hours of deprivation as a simple index of "thirst" and lend support to Miller's conclusions (*4*) about the complexity of drive measures in general.

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4. N. E. Miller (*1*), especially pp. 1275 ff. See also J. M. Mandler's study showing the effect of irregular maintenance schedules and drive measures [*Science* **126**, 505 (1957)].

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Effects of Polyvalent Cobalt Salts on Human Cells in Tissue Culture

Abstract. Trivalent and hexavalent cobalt complexes have been tested to determine their ability to produce morphological changes in two different human cell cultures. Aortic intimal cells which produce mucopolysaccharides in vitro prove to be more susceptible than the nonmucopolysaccharide producing HeLa strain. This effect seems to be related to the reaction of polyanions such as ribonucleic acid, deoxyribonucleic acid, and chondroitin sulfate, with the complex cobalt salts.

The use of polyvalent cobalt salts for the precipitation of chondroitin sulfate and deoxyribonucleate (*1, 2*) prompted us to investigate the effects of these salts on freshly grown cells capable of synthesizing sulfated mucopolysaccharides. Trivalent hexamminecobaltic chloride, $Co(HN_3)_6Cl_3$, and hexavalent hexol nitrate, $[Co(OH)_6(Coen_2)_3](NO_3)_6$, prepared by Maxwell Schubert (*3*), were examined for purity by x-ray powder diffraction patterns (*4*). The trivalent salt in dilute aqueous solutions forms precipitates with sulfated mucopolysaccharides and nucleic acids (RNA as well as DNA), and their respective protein complexes. At physiological *p*H and salt concentrations, only DNA is precipitated (*2*). The hexavalent salt will also precipitate hyaluronate and react with polyanions at physiological *p*H and ionic strength. That the cobalt salt may also form soluble complexes with polyanions is indicated by the dissolution of such precipitates by excess anion, and a specific increment at 235 mμ produced in a solution of hexamminecobaltic chloride by the presence of chondroitin sulfate (*3*).

To test whether these salts could precipitate the mucopolysaccharides or polysaccharide-protein complexes of aortic cells specifically, or both, several human aortas were extracted. Water soluble aortic mucopolysaccharide-protein complex was obtained by Schubert's method (*5, 6*). This material was metachromatic, had a clear absorption peak at 280 mμ in a Beckman DU spectrophotometer, and contained 9.8 percent hexosamine and 8.2 percent nitrogen. It was obtained in a yield of 1 to 2 percent of dried, defatted aorta and sedimented as two peaks in the analytical ultracentrifuge. In aqueous solution the material gave precipitates with both polyvalent cobalt salts directly proportional to the amount of polysaccharide-protein complex added.

Due to the relative specificity of the reaction, we proposed to study whether these salts would (i) enter cells growing in tissue culture and (ii) manifest effects either in cytoplasmatic sites of RNA and mucopolysaccharide localization or in nuclear DNA itself. Two human cell types were used: (i) intimal cells from adult human aorta organ cultures (*7*) which produce mucopolysaccharides in short-term studies (*8*), and (ii) a commercial stock of strain HeLa (*9*) (Microbiological Associates, Inc.). Both cell types were maintained in 10 percent human serum and 90 percent Eagle's basal medium for periods of up to 120 hours.

The cobalt salts were dissolved in triple distilled water with a magnetic stirrer, sterilized by filtration through a UF fritted disc (Corning) in a Morton filter apparatus, and diluted to concentrations of 1 to 150 μg/ml in sterile Hanks' solution. After the drug was added, flying-coverslip type cultures were incubated at 36.5°C and observed periodically. The cultures were fixed in 10 percent Ringer's formalin and stained with May-Grünwald-Giemsa by W. Jacobson's method (Strangeways Laboratory), Alcian blue 8 GS-Ehrlich's hemalum, and periodic acid-Schiff.