treatment. While the mutational effect increased with increased chemical concentration, at least between the two higher concentrations, neither sterility nor lethality patterns were the same. Sterility was manifested only at the highest concentration of diepoxybutane, while even a partially lethal dose was not demonstrated with any of the concentrations employed. At the same time, two previously unreported effects, chlorophyll-deficiency sectoring and delayed germination, were produced by diepoxybutane treatment.

All of these effects, excluding the mutational effect, were restricted in tomato to the 0.8 percent diepoxybutane treatment. In addition, chlorophyll-deficiency sectoring in first true leaves and delayed germination were relatively constant features of this treatment.

The constancy of delayed germination and chlorophyll sectoring in the 0.8 percent diepoxybutane treatment indicates possibly that there is a relatively invariable response of the individual tomato seeds in a treatment to a given concentration of chemical. And, too, if one assumes these effects to be the result of gross chromosomal aberrations, the ratio of normal to damaged meristematic cells in the seed treated with 0.8 percent diepoxybutane has reached the threshold point necessary for the manifestation of these effects. On the other hand, if lethality is dependent on all meristematic cells of a seed being irreparably damaged, then the dosage necessary for this condition had not yet been administered in this experiment.

The results of this study may thus indicate the tomato to be a valuable material on which to study the quantitative effects of chemical mutagens in higher plants. First, diepoxybutane has demonstrated mutagenic effects over a wide range of chemical concentrations (0.2 to 0.8 percent) without being limited by lethality or sterility. Second, the seed itself demonstrated few properties that would cause variations in the effects of a given concentration of chemical with an individual treatment (*4*).

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26 February 1960

Effect of Synthetic Smog on Spontaneous Activity of Mice

Abstract. Mice allowed to run in a revolving-drum activity cage are sensitive indicators of air pollution. They respond to the presence of synthetic smog by diminishing their 24-hour activity in the revolving wheels. The reduction in wheel activity is comparatively greater for larger amounts of smog.

Since Stewart's pioneering experiments on the measurement of the activity of rats and mice with revolving wheels and kymographs, numerous investigators have studied the factors which influence the behavior of rats in this type of apparatus (*1*). Attempts have been made to calibrate or improve the wheels, or invent new means of measurement, such as tambour-mounted or tilting cages, photoelectric or magnetic devices, and a variety of mazes, most of which measure different quantities (*2*). Furthermore, many environmental and biological factors affect activity, yet relatively little is known about the motivation involved, despite several investigations of this phase of the problem (*3*).

Some of the factors which tend to complicate the use of these techniques are age, sex, diurnal cycle, oestrus cycle, visible light (*5*), heredity (*6*), and hunger and dietary deficiencies (*7*). Drugs with both stimulating and depressing effects on activity are known (*8*). Whole-body radiation also exerts some influence, though activity is not especially responsive to this kind of insult (*9*). Tobacco smoke is also claimed to have some effects (*10*). Although other air pollutants have not been systematically studied, one of Stewart's original observations is of interest in this connection. During the course of his experiments on the effects of barometric pressure and alcohol on the activity of rats, he observed a decrease on several occasions which he attributed to the escape of gas in his laboratory. While he does not indicate whether the poisoning seriously affected his animals in other ways, he was apparently the first to observe the effect of an air pollutant on voluntary activity (*1*).

The experiments described in this report were performed in the expectation that biological methods which measure the voluntary behavior of the experimental animal would provide sensitive indicators of environmental factors such as air pollution and infectious agents, since relatively small sensory impulses may be amplified by the neuromuscular system of the animal into large changes in behavior (*4*).

For the purpose of studying these effects, we have employed two modified 100 ft^3 refrigerators. The chambers are similar and are provided with activated charcoal filtered air pulled by an exhaust blower on the roof. The temperature is approximately the same in both chambers. A mixture of ozone and gasoline vapor in air is forced into the exposure chamber. (The technique is similar to that described by Kotin and Falk, *11*.) This smog is analyzed daily for total oxidant with phenolphthalein (*12*) and for ozone by absorption in neutral potassium iodide (*13*).

The mice used for the present study were young adult C57BL/6 males; they were caged individually, and they had

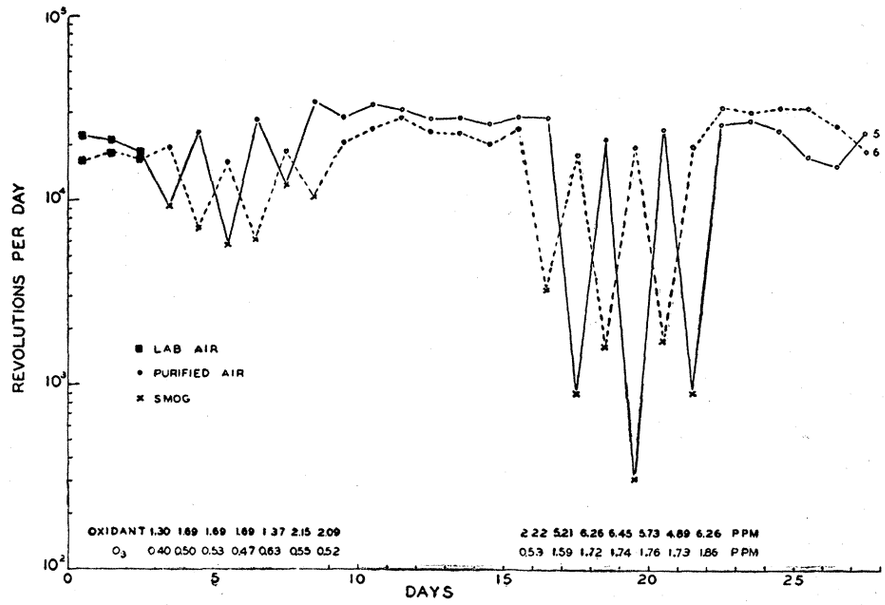

Fig. 1. Spontaneous wheel turning activity of two C57 Black male mice in different environments. The total oxidant and ozone determinations are shown at the bottom of the graph for each day of exposure to synthetic air pollutant mixture.

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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24 December 1959

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