to the altered susceptibility by differences in adsorption or penetration of virus or in production of interferon was offered.

Summary. Cell cultures of bovine embryonic lung were found to undergo morphological changes after inoculation with polyoma virus. The changes were characteristic for polyoma transformation with cells having stellate or triangular shape and lying at random, crisscrossing one another.

Cell lines of rapidly-growing transformed cells were obtained. Attempts to isolate infectious virus from the lines were negative. The lines contained "tumor" antigen as demonstrated by complement fixation tests using serum from hamsters bearing polyoma-induced tumors.

A comparison of the viral susceptibility between the polyoma-transformed cells and SV40-transformed and normal cells derived from the same source revealed certain differences. Whereas the polyoma-transformed cells showed fairly high susceptibility to a wild strain of type C of foot-and-mouth disease virus (FMDV) the SV40-transformed and normal cells were less susceptible. The SV40-transformed cells showed a decreased susceptibility to bovine enterovirus (BEV) and pseudorabies virus (PRV) in comparison with the polyoma-transformed and normal cells.

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Influence of Environmental Temperature on Resistance to Endotoxin. (31964)

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It has been reported that mice stressed by an environmental temperature of 37°C, when compared with non-stressed mice at 22° or 23°C, exhibit an increased mortality to Gram-negative bacteria(1) or their extracts(2), including endotoxin(3). It is not known, however, if mortality varies with environmental temperature within a temperate and non-stressful range. The present report describes such variation within the range 18° to 33°C in mice injected with endotoxin.*

* This work was presented at the meeting of the Federation of American Societies for Experimental Biology: Fed. Proc., 1967, v26, 583.

Materials and methods. Constant temperature rooms. Well-ventilated rooms, about 4.5 × 3 × 2 m^3, were each kept at a constant temperature, ± 1°C, by means of an adjustable heating unit, an adjustable cooling unit, and a thermostat. Each room was kept at one of the following temperatures: 18°, 22°, 27.5°, 31.5°, and 33°C. (It has been reported that in environments below 18°C, non-treated mice develop subnormal body temperatures, while in environments above 33°C, they develop fever(4). Consequently, we did not make observations either below 18° or above 33°C. Furthermore, we have observed

TABLE I. Number of Dead Mice in Each Group of 4 Animals.

Dose, μg/g	At end of 24 hr					At end of 48 hr*				
	18°	22°	27.5°	31.5°	33°	18°	22°	27.5°	31.5°	33°
0				0	0					Unchanged
5	0			1	2	0				
10	0	0	0	1	2	0	0	0		from the
15	1	0	0	2	4	1	0	0		
20	0	0	1	0	4	0	0	2		end of
25	1	0	3	4	4	3	0	4		
30	2	1	2	2	4	2	1	3		24 hr
35	3	3	1	4		3	4	3		
40	2	2	3	3		3	3	3		
45	2	1	3	4		3	2	4		
50	4	4	4	4		4	4	4		
55	4	1	3	4		4	2	4		
60	4	3	0			4	4	3		
65	4	3	3			4	4	4		
70	4	4	3			4	4	4		
75		4	4				4	4		
80		4	4				4	4		
85		4					4			
90		4					4			
LD_{50}	33.1	45.7	39.2	21.9	7.5	28.4	35.0	23.6	21.9	7.5
S.E.	±3.8	±4.6	±5.4	±3.8	±2.7	±3.5	±3.9	±3.5	±3.8	±2.7

* No additional deaths had occurred at the end of 72 hr.

in non-treated mice an increasing mortality above 33°C, another reason for not making observations on treated mice above this temperature. For example, by the end of 48 hours at 37°C, 5 of 20 normal mice died; in a duplicate experiment, 6 of 20 died.) Air currents, humidity, air pressure, and other climatic conditions were not controlled.

Animals. Adult ♀ ICR albino mice,† 16-30 g in weight, were kept in groups of 20 in cages about 30 × 20 × 15 cm^3, and were permitted to eat and drink at will. They were kept in a local animal room, 22-30°C, for a week after their purchase. They were then transferred to one of the constant temperature rooms and were injected the next day with endotoxin between the hours of 9 a.m. and 11 a.m. They were kept in the constant temperature room for 72 hours after injection.

Endotoxin. Buffered normal saline, pH 7.2, was added in small increments to *S. typhosa* 0901 endotoxin (lot #481100, Difco Laboratories, Detroit, Mich.) until a fine colloidal suspension, concentration 2 mg/ml, was obtained. Several 30 ml samples of the suspension were stored at −20°C until the day of injection. On that day a sample was warmed to the temperature of the room and the mice were promptly injected.

† Gofmoor Farms, Westboro, Mass.

First experiment. The purpose of the first experiment was to determine if the LD_{50} of endotoxin varied with environmental temperature. Each mouse was injected with a dose of endotoxin ranging from 5 μg/g to 90 μg/g body weight in increments of 5 μg/g. Control mice each received 0.5 ml of buffered normal saline without endotoxin. At each dose level 4 mice were injected intravenously through a tail vein. Counts of living and dead mice were made at the end of 24, 48, and 72 hours in order to determine whether a temperature that favored survival in the short run (24 hours) also favored survival in the long run (72 hours). The LD_{50} was determined according to the method of Reed and Muench (5); the standard error, according to the formula of Pizzi(6).

Results of first experiment. The LD_{50}'s and their standard errors are given in Table I for the 5 experimental temperatures. Table I clearly reveals that the LD_{50} varied with temperature.

Most deaths occurred in the first 24 hours. At the lower temperatures, additional deaths occurred between 24 and 48 hours, but no further deaths occurred between 48 and 72 hours. Consequently, LD_{50}'s at 48 hours (or

TABLE II. Number of Dead and Living Mice in Each Group of 16 Animals.

Temperature (°C)	At end of 24 hr			At end of 48 hr		
	Dead	Living	Total	Dead	Living	Total
18	5	11	16	6	10	16
22	1	15	16	2	14	16
27.5	5	11	16	10	6	16
31.5	9	7	16	9	7	16
Total	20	44	64	27	37	64

Components of χ^2:	Degrees of freedom	χ^2	P	χ^2	P
22° against 18°, 27.5°, & 31.5°	1	6.0	$.010<P<.025$	7.6	$.005<P<.010$
Differences within 18°, 27.5°, & 31.5°	2	3.7	$.100<P<.250$	3.0	$.100<P<.250$
Overall χ^2	3	9.7	$.010<P<.025$	10.6	$.010<P<.025$

72 hours) were lower than those at 24 hours. In spite of this lowering of the LD_{50} between 24 and 48 hours (or 72 hours), the variation with temperature exhibited at the end of 24 hours did not disappear by the end of 72 hours. Thus, if a temperature favored survival as early as 24 hours after endotoxin injection, that temperature continued to do so at least through 72 hours.

Table I further reveals that at 33°C mice are most susceptible to endotoxin ($p<.05$). Although mice appeared to be least susceptible at 22°C, the experiment did not establish this result to be significant because of the large standard errors in the LD_{50}'s. Therefore, a new experiment was performed.

Second experiment. The purpose of the second experiment was to determine whether at 22°C mice were in fact least susceptible to the lethal effect of endotoxin. Notwithstanding the large number of animals that were used in the first experiment, the number was insufficient to make this determination, because of the large standard error in each LD_{50}. Rather than repeat LD_{50}'s with even more animals, which would have been necessary in order to reduce the standard error, we designed instead a new experiment that could give a significant ($p<.05$) answer with even fewer animals. We noticed that in groups which had received 20-25 μg/g of endotoxin, deaths occurred in all of them except within the group at 22°C (Table I); and we reasoned that this particular dose was the one most likely to discriminate between mortality at 22°C and mortalities at all the other temperatures. Therefore, groups of 16 mice were placed one in each of 4 different constant temperature rooms at 18°, 22°, 27.5° or 31.5°C. (A group at 33°C was omitted because the first experiment had already shown this temperature to be the one most unfavorable for survival.) Each mouse received 24 μg/g, and counts of living and dead mice were made 24 and 48 hours after injection.

Results of second experiment. The results of the second experiment, as shown in Table II, indicate that the variation of mortality with temperature was significant ($p<.025$). Furthermore, susceptibility at 22°C was indeed significantly lower ($p<.025$) than that at any other temperature.

Summary and conclusions. We have measured the mortality of mice injected with endotoxin at 5 environmental temperatures ranging from 18° to 33°C. At environmental temperatures 20-25°C mice are most resistant to endotoxin, while at temperatures both above and below this range they are increasingly susceptible to it.

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Radiation Sensitivity of the Hibernating Ground Squirrel, *Citellus tridecemlineatus.** (31965)

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The sensitivity of hibernating and active ground squirrels, *Citellus tridecemlineatus*, to ionizing radiation (Co^{60} photons) has been the subject of study in our laboratory. In the course of investigations dealing with radiation effects on intestinal absorption in the ground squirrel(1,2,3), we noted that many hibernating animals exposed to lethal levels of radiation survived post-arousal periods of one month and longer, whereas animals irradiated in the active state died much sooner. The increased survival periods of irradiated hibernating squirrels after arousal as compared to the post-irradiation survival periods of active squirrels were not in accord with the findings of others(4,5,6,7). The consensus has been that upon arousal irradiated hibernating animals react the same as irradiated non-hibernating subjects. These apparent differences prompted the present study.

The objectives of the experiment herein described are: Is there a difference in the sensitivity of hibernating and non-hibernating ground squirrels to various high levels of radiation and is there sufficient reason to suspect that radio-protection occurs in a hibernating mammal?

Materials and methods. Active and hibernating ground squirrels. Seventy-two animals for experimentation were taken from a colony of stock animals. The animals were young of the year, *i.e.*, from litters produced during late Spring, 1965. Specimens were selected at random, and both males and females, circa 150 to 200 g, were used. From the same stock colony, a group of 12 animals have been maintained in an identical manner and have served as controls for long term comparisons, *e.g.*, to assess longevity and ageing changes.

Squirrels in the hibernating group were placed in an environmental chamber at 5° to 6°C for periods averaging about 3 weeks. The individual hibernacula were plastic containers (transparent lucite cylinders 19 cm high and 14 cm in diameter containing about 7 cm of San-i-cel corn cob bedding). During the hibernal period frequent daily observations were made to determine phases of arousal in each animal; in this way only those animals in deep hibernal torpor were chosen for irradiation.

Irradiation of animals. During the irradiation procedure animals were individually housed in plastic containers (described above) and each active and hibernating animal was irradiated singly. In order to minimize possible effects of circadian and/or circanian rhythms all animals were irradiated between 2 PM and 5 PM CST during the months of February and March, *i.e.*, during the usual seasonal period of hibernation.

Representatives from each group were given whole body exposure to radiation doses ranging from 1000 r to 3000 r. The dose rate was 200 r per minute and was considered constant over the entire period of the experiment.

The irradiation was conducted by means of a GR-12 Gamma Cell built by United Nuclear of California. The radiation source consisted of 6 stainless steel "pencils" (20 cm in height, equally spaced around the cylindrical sample chamber 15 cm in diameter) in which the Co^{60} loading was approximately 150 curies. The Co^{60} was arranged in the pencils such that the photon flux over the volume of the sample chamber varied less than 5%. Dosimetry was obtained by the ferrous-ferric sulphate technique(8) and a 250 r Victoreen ionization chamber.

Immediately following the irradiation of a hibernating squirrel, rectal temperature

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