TABLE I. Alteration of Survival Time in Ehrlich's Ascites Mice Produced by Cyanide-Anesthesia Treatment.

Group No.		Dosage (mg/kg)	Cells/mouse	Avg survival (days)	Increase in survival (days)	% increase
I	Control	.75	8×10^7	6.2	0	0
	5 days	"	"	8.8	2.6	42.0
II	Control	.75	4×10^6	8.1	0	0
	24 hr	"	"	9.8	1.7	21.0
	120 "	"	"	10.4	2.8	35.0
III	Control	1.5	"	8.1	0	0
	24 hr	"	"	12.7	4.6	56.8
	48 "	"	"	13.8	5.7	73.7
	120 "	"	"	11.1	3.0	37.0
	27, 72, 120 hr	"	"	12.8	4.7	64.1

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Adrenal Cortical and Body Temperature Responses to Repeated Endotoxin Administration. (24944)

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It is difficult to speculate logically on the mechanism of adrenal cortical stimulation following endotoxin, inasmuch as fever and hypotension may be responsible. Previous studies indicate that the adrenal cortical stimulating effect occurs before fever(1), but most animals that reveal pituitary-adrenal activation following endotoxin also develop fevers. The present studies were set up to produce tolerance to fever-promoting effect of endotoxin. The ease of producing tolerance to various responses following endotoxin varies considerably, and it was possible that fever-tolerance might occur before loss of pituitary-adrenal stimulation.

Methods. Adult mongrel dogs ranging 8-25 kg were used. Cannulation of right lumbar vein(2) was performed according to the technic of Hume and Nelson, and intermittent samples of adrenal venous blood were obtained. Cortisol concentrations in the adrenal venous blood were determined by modification of method of Silber and Porter(3) as described by Peterson, Karrer and Guerra(4). Purified endotoxin of Boivin type derived from *E. coli* was prepared by method of Spink and Anderson(5). Endotoxin was prepared in 2 concentrations in normal saline, one of 1 mg/cc, the other 0.1 mg/cc. All endotoxin injections were by intravenous route and given in approximately 30 seconds time. Twenty-five units of ACTH were given intravenously at conclusion of some experiments. Rectal temperatures were taken, and in some experiments arterial blood pressures were obtained by cannulating femoral artery with polyethylene catheter connected to mercury manometer.

Results. The adrenal cortical and fever responses to large doses of endotoxin are given in Table I. Significant adrenal cortical responses were observed in all animals except

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TABLE I. Effect of Repetitively Administering Large Doses of Bacterial Endotoxin on Body Temperature and Adrenal Cortical Function.

Exp. No.	Days of previous endotoxin exposure	Dosage of endotoxin (mg)	Adrenal venous blood 17-hydroxycorticosteroids (μg/min.) Time after endotoxin (min.) Control	5	60	120	180	Rectal temp. elevation (°C)
R-1	3	4.0	1.8	10.0	16.3	15.8	1.1	1.9
R-2	5	4.0	1.6	11.4	10.2	5.3	11.6	2.5
R-3	3	.5	.7	2.7	6.9	1.9	1.6	2.2
R-4	3	.1	.7	2.2	1.0	.9	1.5	.3
R-5	3	.1	.3		6.0	5.7		N.D.*
R-6	3	.1	3.2	3.6	5.4	7.6	9.9	2.2
R-7	3	.1	.5	.8	5.6	5.2		N.D.
R-8	3	.1	.7	15.9	13.1	15.1	1.7	2.0

* N.D.—not determined.

R-4, with dosages ranging 0.1 to 4 mg of endotoxin/day, and for periods to 5 days. Except for R-4, no instance of tolerance to fever-stimulating effects of endotoxin were observed.

In Table II are given results following administration of 0.01 mg of endotoxin for 1 or more days prior to collecting adrenal venous blood samples. In none of the 8 experiments was an elevation in rectal temperature or hypotension observed, although all animals had rectal temperature elevations of at least 1.5° and significant adrenal cortical responses on first day of endotoxin administration. Although all animals manifested tolerance to fever-stimulating effect of endotoxin, only one animal, ER-15, was completely tolerant to adrenal cortical-stimulating effect. All other animals demonstrated significant adrenal cortical responses following administration of endotoxin, although this response was not maximal when compared to that following intravenous ACTH. In some experiments the differential between maximal levels of cortisol output following endotoxin and that following ACTH was less than in others.

Experiments were then performed in which endotoxin was administered on consecutive days, with time intervening before final injection, Table III. In ER-1, a maximal adrenal cortical response and significant fever was observed following administration of endotoxin. On second day of endotoxin administration a significant adrenal cortical effect was observed, but no fever. At 60 days fever tolerance had been lost. Similar findings were observed in Exp. ER-4 and ER-5, in which fever responses returned at 43 and 36 days respectively. In Exp. ER-12 fever tolerance existed on second and third day following administration of 0.01 mg of endotoxin, but 3 days later normal fever response had been restored, Fig. 1. Adrenal cortical response was significant on days 2 and 3, in absence of fever. The response was not maximal as shown by increase in corticoid output follow-

TABLE II. Effect of Repetitively Administering 0.01 mg of Bacterial Endotoxin on Body Temperature and Adrenal Cortical Function. All animals had rectal temperature elevations of at least 1.5°C and significant adrenal cortical responses on 1st day of endotoxin administration.

Exp. No.	Days of previous endotoxin exposure	Adrenal venous blood 17-hydroxycorticosteroids (μg/min.) Time after endotoxin (min.) Control	60	90	120	5 min. after I.V. ACTH	Rectal temp. elevation (°C)
ER- 2	1	1.3	11.9		9.5	14.4	+.6
ER- 3	1	.2	4.6	6.1	7.1	14.0	−.1
ER- 6	1	3.7	9.5	12.0			No change
ER- 7	1	1.8	6.7	6.1	6.0	14.1	+.2
ER-14	5	.2	6.3	8.9		18.3	+.4
ER-15	5	.5	.2	1.8	.5	9.1	No change
ER-16	6	.4	3.8	4.8	6.8	13.9	"
ER-17	6	.4	3.6	8.9	9.2	25.8	"

TABLE III. Adrenal Cortical and Body Temperature Responses of Dogs Given 0.01 mg of Endotoxin on Consecutive Days, Followed by Later Endotoxin Administration.

		Adrenal venous blood 17-hydroxycorticosteroids (μg/min.)					
	Day of endotoxin	Time after endotoxin (min.)				5 min. after	Rectal temp.
Exp. No.	admin.	Control	60	90	120	I.V. ACTH	elevation (°C)
ER- 1	1	1.9	10.5	10.4	11.6	8.1	+1.6
	2	.4	8.1			7.1	- .2
	60	.7	9.6	11.1			+1.8
ER- 4	1	.3	7.5	7.7	13.7	12.5	+1.6
	2	1.2	4.5	2.4	10.0	8.5	+ .2
	43	.4	5.2	8.1	9.8	8.5	+4.0
ER- 5	1	2.7	3.9	6.1	5.6	8.1	+ .9
	2	1.4	3.7	6.7	6.0	12.6	- .3
	36	.6		4.5	6.1		+2.9
ER-12	1	.8	9.1	8.0	16.8	14.4	+2.8
	2	.4	4.5		8.8	19.3	+ .1
	3	.3	9.7	7.9	9.0	13.6	+ .4
	6	.2	6.6	11.5	13.8	16.7	+3.1

ing administration of intravenous ACTH. Normal fever and adrenal corticoid responses had returned by 6th day.

Discussion. Our results indicate that tolerance to fever-promoting effects of endotoxin may be induced more readily than to adrenal cortical stimulating properties. In many experiments partial tolerance to adrenal cortical stimulating properties occurred, as demonstrated by failure of endotoxin to cause maximal adrenal cortical stimulation when compared to intravenous ACTH. However, some animals which became tolerant to fever stimulating properties retained a normal maximal adrenal cortical response to endotoxin (Table III). It appears, therefore, that varying degrees of tolerance to adrenal cortical stimulating effect occur in the dog.

Fever tolerance in dogs in doses of 0.01 mg of endotoxin is very short lived and several days after cessation of endotoxin administration normal fever responsiveness occurs. Our experiments certainly establish that neither fever nor hypotension are prerequisites to adrenal cortical stimulating effects of endotoxin, a conclusion suggested by previous findings that adrenal cortical stimulation precedes appearance of fever in dogs given an initial dose of endotoxin(1). We do not conclude that partial adrenal cortical tolerance is due to an effect on adrenal, pituitary or elsewhere. That it is not the adrenal cortex seems likely because of normal responsiveness to exogenous ACTH. This is quite different from decreased responsivity to exogenous ACTH following larger quantities of endotoxin as previously described(1). It is quite possible that "partial tolerance" represents nothing more than decreased ACTH release by the pituitary. This possibility is at present being evaluated.

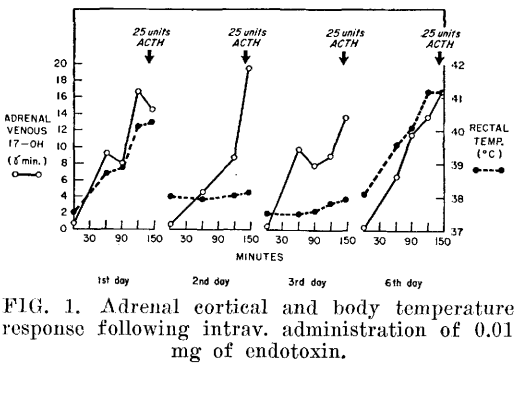

FIG. 1. Adrenal cortical and body temperature response following intrav. administration of 0.01 mg of endotoxin.

There is a precedent for the tolerance observed on second day of endotoxin administration. Bernhimer and Cantoni(6) found that mice became temporarily resistant to lethal effect of a streptococcus toxin after one injection of toxin, and that resistance was present 3-6 hours after initial dose and was gone by 40 hours. With regard to the mechanism of release of ACTH following endotoxin, spinal cord section does not prevent the response(7). Endotoxin is not a corticotrophin-like substance since it does not result in adrenal cortical stimulation in the hypophy-

sectomized animal. Accordingly it is proposed that endotoxin acts directly on the central nervous system to result in ACTH release. Experiments have been undertaken to determine site of action of endotoxin in the central nervous system for both adrenal cortical stimulating and fever-promoting properties.

Summary. Dogs given large doses of bacterial endotoxin do not become tolerant to either fever producing or adrenal cortical stimulating effects. Dogs receiving small doses of endotoxin readily become tolerant to the fever-producing effect, but either do not become tolerant at all or achieve only partial tolerance to adrenal cortical stimulating properties. The partial adrenal cortical tolerance observed following endotoxin administration probably represents decreased pituitary release of the ACTH rather than tolerance of adrenal cortex itself.

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A New Type of Lysis from Without. (24945)

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According to the enzyme hypothesis of phage action on susceptible bacterial cells(1-6), holes are made in bacteria cell wall, by phage enzyme following adsorption, and nucleic acids of the phage are injected through these holes into the cell. Within a few minutes after adsorption these holes are sealed (3), provided the damage to cell walls is not too great, as in lysis from without(1). One might therefore expect that if the phage enzyme acted on bacteria which were blocked in their ability to reseal even the minor "tears" in their walls, initial lysis would not be halted and the enzymatic action would disintegrate the wall completely. Such immediate lysis caused by T2r phage was observed by Heagy(7) in partially starved *Escherichia coli*. The present study shows that freezing and thawing of exponentially growing bacteria provides a shock which blocks repair of damage to bacterial wall, and that such thawed bacteria lyse immediately upon addition of the phage.

Methods. E. coli, strain B or B/r, was grown in broth or in a synthetic medium(8) until an optical density of 0.4-0.6 (at 540 mμ) was reached. This corresponded to a viable count of 2 to 4 x 10^8 cells/ml. The suspension was either directly frozen in the growth medium, using a CO_2-acetone bath, or it was washed by centrifugation and frozen in fresh medium. The bacterial suspensions were infected with various bacteriophages (T2r$^+$, T4D, T4Dr47, T3, or T7), either before freezing or after thawing. In the first case, phage was added to cells at a desired multiplicity of infection, the suspension incubated 3 min. at 37°, and frozen. In the second type of experiment, phage was added immediately upon thawing, *i.e.,* at moment of final disappearance of ice. The volume of added phage was 1/30 to 1/50 of the bacterial suspension, while multiplicity of infection varied between 1 and 15. For determination of onestep growth curves, unadsorbed phage was removed by centrifugation.