THE EFFECT OF BACTERIAL ENDOTOXINS ON THE WATER INTAKE AND BODY WEIGHT OF MICE

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One of the many effects of the injection of bacterial endotoxins into experimental animals is a profound, albeit transient loss of body weight. As previously reported, this weight loss occurs even with mice of the new NCS colony, although these animals are remarkably resistant to the lethal effects of endotoxins (1, 2). More recently we have observed that mice stop drinking for many hours after receiving endotoxins and this suggested that the weight loss caused by these materials might be due in part at least to the dehydration resulting from decreased water intake. Several related aspects of these phenomena are described in the present paper.

Methods

The origin of the cultures of *Escherichia coli* 17, *Klebsiella pneumoniae*, *Staphylococcus aureus* (strain Giorgio), and *Mycobacterium tuberculosis* (BCG-P) and their maintenance have been described in preceding publications (1–4). Endotoxins from cultures of *Esch. coli* 17 and *Klebsiella pneumoniae* were prepared in our laboratory by the techniques described earlier. Two other preparations of endotoxin were used. One is a commercial product prepared from a culture of *Esch. coli* (lot control 439277 from Difco Laboratories, Inc., Detroit). The other was prepared from *Salmonella abortus equi* by Dr. Otto Westphal who generously supplied us with a highly purified sample. (Dr. Westphal designates this material pyrexal.)

The mice were animals of the new NCS colony, maintained as previously described in a state free of ordinary mouse pathogens as well as of intestinal *Proteus* bacilli and *Esch. coli* (1).

The diet consisted either of (*a*) commercial pellets obtained from Dietrich and Gambrill (D & G), Frederick, Maryland; this diet is almost completely bacteria-free; or (*b*) corn grain ground in water and supplemented with 4 per cent (dry weight) of Wesson's salt mixture.

For the measurement of water intake the mice were kept either single, or in groups of 3, 4, or 5 per cage. The water consumed was determined by weighing the water bottles at intervals of time; all figures reported in the present paper refer to water consumption calculated in milliliters per mouse.

All injections of bacterial vaccines or endotoxins were by the intraperitoneal route, the materials being injected in a final volume of 0.2 ml. of pyrogen-free saline (Abbott Laboratories, North Chicago).

RESULTS

1. Water Intake of Normal NCS Mice.—The water intake of NCS mice was repeatedly measured at different hours of the day. For a given diet it remained

constant within narrow limits from day to day but it varied with the diet and exhibited a marked diurnal cycle. The range of variation is illustrated in Table I which presents results from an experiment selected because it exhibited differences in daily water intake larger than those usually encountered. It is seen that male mice 5 weeks old and weighing from 18 to 23 gm. drank 4.5 to 6.3 ml. per 24 hour period when fed the D & G diet, and only 3.3 to 4.3 ml. when fed ground corn supplemented with minerals.

With both diets, normal animals hardly consumed any water during the daytime and began drinking only around 8:00 p.m. (Table I). This diurnal

TABLE I

*Water Intake of Normal Mice**

Time interval (3 consecutive days)	Water‡ fed as indicated					
	D & G diet			Corn		
9:30 a.m. to 7:00 p.m.	0.3‡	0.1‡	0.4‡	0.‡	0.1‡	0.2‡
7:00 p.m. to 9:30 a.m.	4.8	5.3	5.0	3.7	3.3	3.5
9:30 a.m. to 7:00 p.m.	0.4	0.2	0.3	0.	0.2	0.
7:00 p.m. to 9:30 a.m.	5.4	4.9	4.2	3.9	4.1	3.3
9:30 a.m. to 7:00 p.m.	0.3	0.5	0.2	0.3	0.1	0.
7:00 p.m. to 9:30 a.m.	5.3	5.0	6.1	3.6	3.4	3.7

* NCS males, 5 weeks old, weighing 18 to 23 gm. Observations made December, 1960, on animals kept in laboratory; lights on during the day, off at night.

‡ Figures refer to individual mice, 3 per diet.

rhythm was disturbed by injection of endotoxin. It must be emphasized that all the observations reported in the present paper have been made on NCS mice maintained in a crowded laboratory during the fall and early winter. Although similar results have been obtained with another strain of mice (Bagg), the findings may not be applicable to other conditions and animals of other colonies.

2. Endotoxin, Water Intake, and Weight Change.—

Male mice 5 weeks old were distributed at random into 12 groups of 3 animals each and were housed in metal cages with wood shavings as litter; they were given D & G diet and water *ad lib.* except as indicated below (See Table II). The animals in 6 boxes received 100 μg. of *Esch. coli* endotoxin (lot control 439277, Difco Laboratories) in 0.2 ml. saline by the intraperitoneal route (cages 1a, 1b, 1c, 3a, 3b, 3c) (Table II). This treatment was given at 4:30 p.m. The other mice received 0.2 ml. saline (cages 2a, 2b, 2c, 4a, 4b, 4c) at the same time. The animals in 3 boxes of each group were deprived of drinking water for the first 16 hours following treatment, *i.e.*, from 5:00 p.m. to 9:00 a.m. (cages 3a, 3b, 3c, and 4a, 4b, 4c). From then on they received

water *ad lib.* The weight of the animals and the water intake were recorded daily at about 9:30 a.m. (Table II, Figs. 1 and 2).

As seen in Table II, all animals treated with endotoxin lost weight during the 16 hour period following treatment; they did not drink during that period even when water was available to them (cages 1a, 1b, 1c). On the other hand, it must be noted that the weight loss caused by endotoxin treatment was no greater than that observed with animals which did not receive endotoxin but

TABLE II

Weight Changes and Water Intake of NCS Mice*

Cage No.	Average weight	Endotoxin	Drinking water	Weight changes‡ (gm./mouse) at indicated No. of hrs.			Water intake‡ (ml./mouse) after injection of endotoxin		
				0 to 18	18 to 42	42 to 66	0 to 18	18 to 42	42 to 66
	gm.	*μg.*							
1a	21.3	100	ad lib.	−1.7	2.2	1.6	0.4	5.0	6.4
1b	19.0	"	" "	−1.8	2.5		0.1	5.0	6.3
1c	16.9	"	" "	−1.4	1.2	0.6	0.2	2.9	4.1
2a	21.0	0	" "	1.7	1.3	1.1	6.1	6.7	7.4
2b	19.0	"	" "	1.5	2.1	1.2	4.7	5.8	6.0
2c	17.0	"	" "	1.4	1.3	1.2	4.5	5.3	6.2
3a	21.0	100	Withdrawn for first 18 hrs., then given *ad lib.*	−2.3	2.2	1.5		5.7	5.6
3b	18.7	"		−1.7	1.8	1.9		4.6	6.0
3c	16.9	"		−1.7	1.5	0.8		3.5	4.1
4a	21.0	0		−2.2	0.8	0.9		8.3	5.9
4b	17.9	"		−2.0	1.1	1.3		8.0	5.8
4c	16.9	"		−1.8	1.2	1.3		7.9	6.6

* Males, 5 weeks old, fed D & G diet *ad lib.*

‡ All figures correspond to averages for 3 mice.

were deprived of water for 16 hours. (groups 4a, 4b, 4c) Taken together these facts suggest that interference with water intake did account in part at least for the weight differences observed between endotoxin-treated and control animals. This interpretation is supported by the finding that the groups of animals deprived of drinking water (3a, 3b, 3c, and 4a, 4b, 4c) lost the same amount of weight whether or not they had received endotoxin. In other words, it appears as if endotoxin *per se* did not increase the weight loss beyond that resulting from dehydration.

When drinking water was restored to the animals which had been deprived

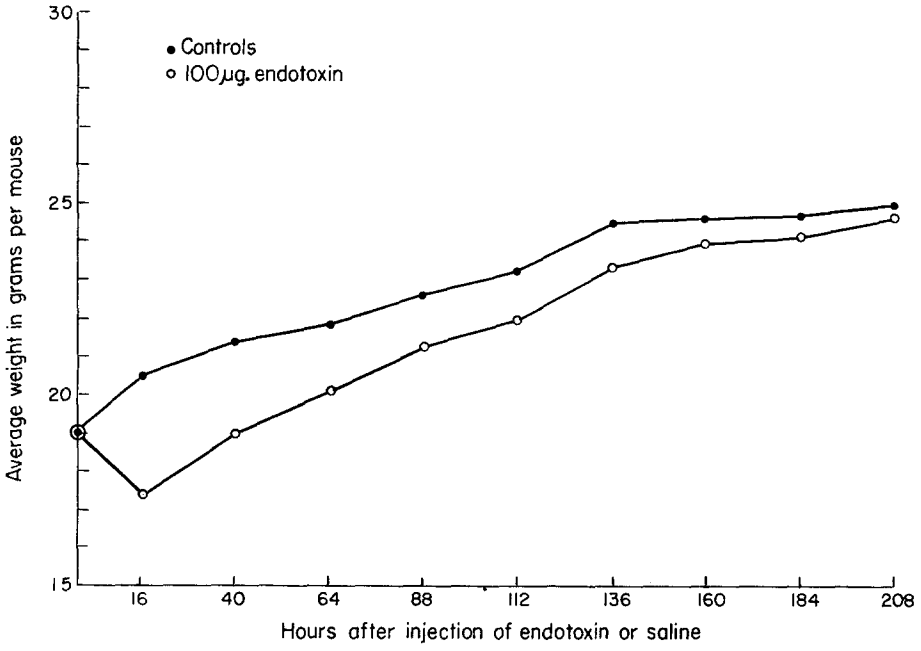

FIG. 1. Cumulative weight changes of mice given water *ad lib.* throughout experimental period. Treated mice received 100 μg. of endotoxin at time 0. Control mice received saline.

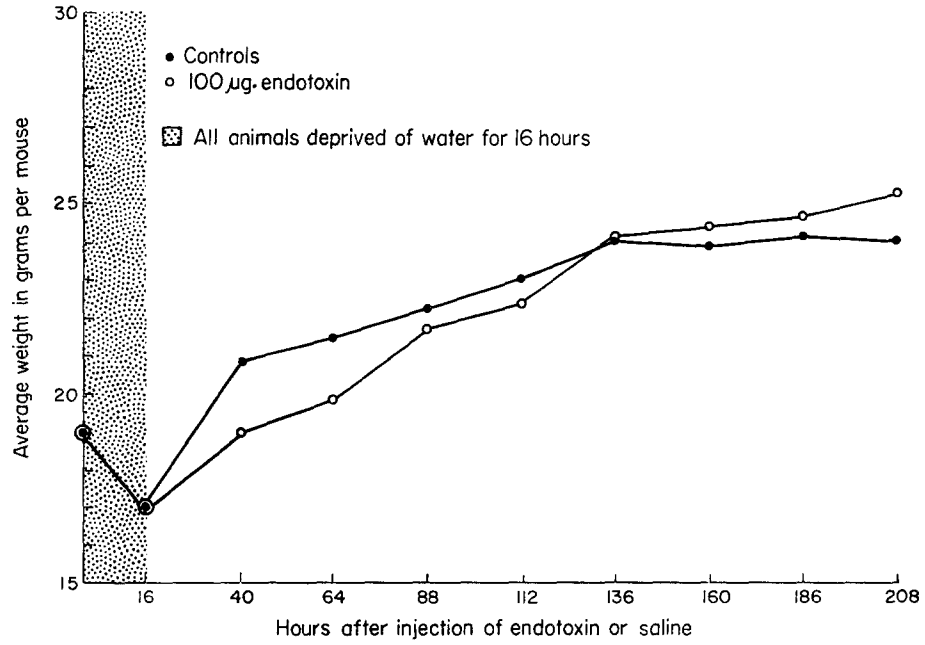

FIG. 2. Cumulative weight changes of mice deprived of drinking water during the first 16 hours of the experimental period, then given water *ad lib.* Treated mice received 100 μg. of endotoxin at time 0. Control mice received saline.

Notice that control mice regained the expected weight level within 24 hours after being given water (compare with Fig. 1), whereas endotoxin mice remain underweight for approximately 5 days (as do endotoxin mice shown in Fig. 1).

of it for 16 hours, the intake during the following 24 hours was much smaller in those having received endotoxin (3a, 3b, 3c) than in those having received saline (4a, 4b, 4c). In fact water intake in all the endotoxin-treated mice remained below that of the control for at least 4 days and it took approximately 1 week before their body weights had become equal to that of the other 2 groups. In contrast, the animals deprived of water but not receiving endotoxin gained weight as soon as water was restored to them (groups 4a, 4b, 4c)—evidence that the prolonged loss of body weight observed in groups 1a, 1b, 1c, and 3a, 3b, 3c was due to the persistent effect of the toxin and not to the initial water deprivation.

The water intake and the weight changes were very similar in the triplicates of each group. This uniformity of the animals with regard to both water intake and its inhibition by endotoxin is the more significant because the groups had been so arranged that for each treatment there were animals ranging from 17 gm. (or less) to 21 gm. (or more) initial weight (see Table II). It seems justified therefore to average the results for the 9 animals of each group as has been done in Figs. 1 and 2 which show the average cumulative weight change plotted for each day of the experiment.

3. Effects of Dose of Endotoxins on Weight Change and on Water Intake.—The findings reported in the preceding section were obtained with a relatively large dose of endotoxin (100 μg.). As seen in Tables III-A and III-B, however, the intraperitoneal injection of very small amounts of endotoxin prepared from either *Esch. coli*, *K. pneumoniae*, or *S. abortus equi* did markedly decrease or indeed interrupt almost completely the water intake of mice. In the experiment presented in Table III-B, 0.03 μg. of endotoxin was sufficient to alter water intake and body weight. In general the effect was detectable within 5 hours and continued for at least 16 hours after treatment. The water intake returned to normal on the following day in the case of animals having received the smallest doses of endotoxin but the inhibition persisted longer as the dose was increased. With 100 μg. of endotoxin, the daily water intake did not return to normal until the 4th day after treatment, as seen in Tables II and VIII. It has been found in many experiments that the inhibition of water intake was essentially the same irrespective of the strain of Gram-negative species from which the endotoxin was prepared.

From the results presented in Table III-A and III-B and those of other experiments not recorded here, it appears that the activity of endotoxin in mice could be titrated in terms of either the minimal amount of material capable of inhibiting water intake, or of the duration of the inhibition caused by a given dose.

4. Correlation between Inhibition of Water Intake and Other Biological Activities of Endotoxin.—The data presented in Tables III-A and III-B make it clear that the inhibition of water intake constitutes an extremely sensitive test

of the biological activity of endotoxin. The minimal concentrations found effective in the experiments reported above are of the same order as those reported by other investigators in tests involving (*a*) excretion of urinary nitrogen following administration of ACTH (6), (*b*) the metabolism of rabbit

TABLE III-A

Early Effect of Two Endotoxins on Water Intake

Endotoxin source		Water intake* (ml. per mouse) at indicated hrs.		
		0 to 5	5 to 18	18 to 42
	μg.			
Esch. coli	10	0.1	0.8	6.6
" "	3	0.4	2.8	4.2
" "	1	0.8	3.2	4.1
K. pneumoniae	10	0.1	1.7	4.1
" "	3	0.5	3.4	4.6
" "	1	0.7	3.0	5.4
Saline		1.2	4.8	4.3

* All animals were NCS male mice, 5 to 7 weeks old, fed D & G diet and water *ad lib.* The figures correspond to averages of three independent experiments involving 12 animals for each dilution of endotoxin.

TABLE III-B

Effect of Small Doses of Endotoxin on Water Intake and Weight Gain

S. abortus equi endotoxin	Water intake* (ml./mouse)	Weight gain* (gm./mouse)
	(during 42 hrs. after treatment)	
μg.		
1.0	11.5	−0.4
0.3	13.8	0.6
0.1	13.2	0.4
0.03	13.6	1.0
Saline	14.7	1.9

* Averages for 6 mice; males 5 weeks old given D & G diet and water *ad lib.*

polymorphonuclear leucocytes *in vitro* (7), and (*c*) mobilization of exudate cells in the peritoneal cavity of the rat (8).

In a preceding paper, we have reported that addition of very minute amounts of endotoxin to staphylococci enables these organisms to produce a fulminating septicemia following intraperitoneal injection into mice (2). More recently we have observed that the smallest amount of endotoxin that can produce this

infection-enhancing effect is also capable of inhibiting completely the influx of polymorphonuclear leucocytes which is brought about by intraperitoneal injection of staphylococci into mice. In the following experiment, an effort was made to observe these three different types of phenomena in mice treated under the same conditions with one single preparation of endotoxin.

TABLE IV

Comparison of Three Different Biological Properties of Endotoxin

Endotoxin from *S. abortus equi* (i.p.)	Water intake (ml./mouse) 18 hrs. following treatment*	0.1 ml. *Staph. aureus* (strain Giorgia) injected intraperitoneally in admixture with indicated amount of endotoxin	
		Staphylococcal colonies per mouse‡	Polymorphonuclear leucocytes in peritoneal exudates§
μg.			
1	4.7	30	±
0.3	5.0	40	+
0.1	5.4	14	+++
0	6.1	15	+++

* Average of 6 mice.

‡ Animals sacrificed 4 hours after injection of staphylococcus endotoxin mixture; skin, tail, and intestines removed and the carcass homogenized. Figures to be multiplied by 5×10^7 to give the total number of staphylococcal colonies per mouse.

§ Smears of peritoneal exudates were made at the time of sacrifice. Symbol ± indicates mononuclear cells predominate; +++, polymorphonuclear cells predominate.

Male mice 5 to 6 weeks old were given by the intraperitoneal route either saline or endotoxin prepared from *S. abortus equi* (pyrexal) in the amounts indicated in Table IV. 6 animals of each group were used for measurement of water intake. In 5 other animals of each group 0.1 ml. of *Staphylococcus aureus* (strain Giorgio) was added to the endotoxin at the time of injection. These animals were sacrificed 4 hours after infection. The peritoneal exudate was examined microscopically for its content of polymorphonuclear leucocytes and the total numbers of staphylococci in the carcass were determined by the method previously reported (2). (Table IV).

As seen in Table IV, 0.3 μg. of endotoxin but not 0.1 μg was capable in this particular experiment of inhibiting water intake, preventing influx of polymorphonuclear leucocytes, and enhancing staphylococcal infection.

5. Tolerance Induced by Prior Injection of Endotoxin.—

Mice which had received various amounts of endotoxin prepared from *Esch. coli* or *K. pneumoniae* (Table V-A, V-B, and V-C) were treated 1 or 2 weeks later with an additional amount of 10 μg. of the same materials. The controls consisted of animals which had received saline instead of endotoxin at the time of the first treatment.

As seen in Tables V-A, V-B, and V-C, the depression of water intake caused by 10 μg. of endotoxin was either obliterated or very much decreased in mice

having previously received the same material. The tolerance was still marked 2 weeks after the initial treatment even when the initial treatment had been with an amount of toxin as small as 0.1 μg. (Table V-C).

TABLE V-A

Tolerance to Inhibition of Water Intake by Two Different Endotoxins

Source of endotoxin	First dose	Second* dose	Water intake‡ ml./mouse
	μg.	μg	
Esch. coli	10	10	8.7
" "	3	"	3.9
" "	1	"	4.2
Saline	Saline	"	2.3
K. pneumoniae	10	10	4.8
" "	3	"	4.6
" "	1	"	4.8
Saline	Saline	"	2.4
Saline	Saline	Saline	4.7

* Second dose of endotoxin given 1 week after first.

‡ Intake during 22 hour period following second dose of toxin.

TABLE V-B

Tolerance to Inhibition of Water Intake by Endotoxin

Esch. coli endotoxin (i.p.)	Daily water intake at indicated days following first injection				*Esch. coli** endotoxin (i.p.)	Daily water intake at indicated days following second injection		
	1	2	3	4 to 6		1	2	3
μg.	ml.	ml.	ml.	ml.	μg.	ml.	ml.	ml.
100	0.9	3.8	4.8	6.0	10	4.1	4.1	5.7
10	3.4	6.3	6.6	6.4	"	3.7	3.9	6.6
1	4.2	—	6.2	5.9	"	2.1	4.4	5.3
0.3	5.1	6.7	6.2	6.3	"	1.2	4.6	5.3
Saline	5.6	5.9	6.6	6.2	"	1.3	4.6	6.0
Saline	5.9	5.7	6.3	6.1	Saline	5.8	6.4	6.6

* Second dose of endotoxin administered 2 weeks after first treatment.

6. *Immunological Specificity of the Tolerance Effect.*—

Male mice 4 weeks old were vaccinated by the intraperitoneal route with *Esch. coli* or *K. pneumoniae*. The vaccines consisted of bacterial cells separated by centrifugation from overnight cultures and then heated at 80°C. for 30 minutes. Two doses of vaccine, each containing approximately 0.1 mg. of bacillary material (dry weight) were given at 2 week intervals. 2

TABLE V-C

Induction of Tolerance by Small Amounts of Endotoxin (Pyrexal)

S. abortus equi endotoxin		Water intake‡ (ml./mouse) during period of 18 hrs.	Change in body weight‡ (gm./mouse) after second treatment
First dose (i.p.)	Second dose* (i.p.)		
μg.	μg.		
1.	10	2.4	−0.6
0.3	"	2.2	−0.4
0.1	"	1.9	−0.8
0.03	"	1.4	−1.8
Saline	"	1.2	−1.6
—§	"	1.4	−1.5

* Interval of time between first and second treatment was 2 weeks.

‡ Average of 6 mice. Response of animals to first dose of endotoxin is presented in Table III B.

§ These mice received no treatment whatever at the time when the first dose of endotoxin (or saline) was administered.

TABLE VI

Protective Effect of Vaccination with Killed Bacilli Against Inhibition of Water Intake by Endotoxins

Vaccine*	Endotoxin‡		Water intake (ml./mouse) at indicated hrs. after endotoxin		
			0 to 5	5 to 18	18 to 42
		μg.			
Esch. coli	*Esch. coli*	10	2.0	2.8	4.7
" "	" "	3	1.1	2.5	4.3
" "	" "	1	0.9	3.1	4.4
" "	*K. pneumoniae*	10	0.2	2.4	4.0
" "	" "	3	0.7	2.6	4.7
" "	"	1	0.8	2.2	4.7
" "	Saline	0	0.9	3.5	5.5
K. pneumoniae	*Esch. coli*	10	0.3	2.5	4.2
" "	" "	3	0.4	3.2	4.8
" "	" "	1	0.7	3.0	5.4
" "	*K. pneumoniae*	10	1.5	2.8	4.9
" "	" "	3	1.3	2.5	5.1
" "	" "	1	1.8	2.5	5.0
" "	Saline	0	1.3	2.9	5.4

* Two doses of heat-killed *Esch. coli* or *K. pneumoniae* injected intraperitoneally at 2 week intervals.

‡ Endotoxin injected intraperitoneally 2 weeks after last vaccinating dose.

weeks after the second vaccinating dose the animals were challenged by the intraperitoneal route with various doses of endotoxins prepared from the strains of *Esch. coli* or *K. pneumoniae* used as vaccines. The water intakes following challenge are recorded in Table VI.

As seen in Table VI, vaccination with heat-killed cells of either *Esch. coli* or *K. pneumoniae* markedly increased the resistance of mice to the inhibitory effect of endotoxins on water intake. The protective effect was greater against the toxin prepared from the bacterial strain with which the animal had been

TABLE VII

Effect of Endotoxin and of Heat-Killed Cells of Three Bacterial Species on Water Intake and Weight Gain

Material injected (i.p.)		Weight change (gm./mouse*) 42 hrs. after treatment	Water intake (ml./mouse*) 18 hrs. after treatment
	mg.		
E. coli‡	0.2	−0.2	0.4
" "	0.02	−0.15	0.5
" "	0.002	0.35	0.9
Staph. aureus‡	0.2	0.2	1.2
" "	0.02	0.4	1.7
" "	0.002	0.3	1.8
Myco. tuberculosis‡	0.2	−0.15	0.4
" "	0.02	0.3	2.0
Endotoxin	0.01	−0.1	0.7
"	0.001	0.15	1.4
Saline		0.5	1.9

* 5 week old males, fed D & G diet and water *ad lib.*

‡ Heat-killed cells; approximate dry weight.

vaccinated. It is of interest, however, that while the tolerance exhibited a certain degree of immunological specificity, there was much overlapping between the protective effects of the two vaccines. A similar degree of simultaneous overlapping and immunological specificity has been found previously when NCS mice were sensitized to the lethal effects of endotoxins by vaccination with killed Gram-negative bacilli (2).

7. Effect of Killed Cells of Various Bacterial Species on Water Intake and Weight Gain.—The experiments described in the preceding sections were carried out with endotoxins prepared by various techniques from four different cultures of Gram-negative bacilli. The following experiment was designed to compare the activity of these endotoxins with that of whole bacterial cells of

three different species killed by heat: one Gram-negative (*Esch. coli* 17), one Gram-positive (*Staphylococcus aureus*), and one acid-fast (*Mycobacterium tuberculosis*).

Esch. coli 17 and *Staph. aureus* (strain Giorgio) were grown overnight in meat infusion peptone broth; *Myco. tuberculosis* (strain BCG-P) was grown in tween-albumin medium for 7 days. The cells were separated by centrifugation, resuspended in pyrogen-free saline, and heated at 80°C. for 90 minutes. Appropriate dilutions of the bacterial suspensions were injected by the intraperitoneal route and the effect on weight of the animal and on water intake recorded as usual. (Table VII).

TABLE VIII

Effect of Diet on Duration of Inhibition of Water Intake by Endotoxin

Diet *ad lib.*	*Esch. coli* endotoxin (i.p.)	Water intake (ml./mouse) following injection of endotoxin at indicated time (in days)					
		1	2	3	4	5	6 to 7
	μg.						
D & G diet	100	0.4	4.6	5.6	6.0	5.4	5.6
" "	10	2.6	6.3	6.1	6.1	5.6	5.9
" "	1	4.9	6.0	6.5	6.4	5.1	5.5
" "	0.3	4.3	6.2	6.2	6.4	5.1	4.8
" "	Saline	5.8	6.1	6.7	6.7	5.2	5.6
Corn	100	0.1	2.4	2.9	2.7	1.8	3.3
"	10	1.7	4.5	4.0	4.8	3.1	3.2
"	0.3	2.7	4.0	3.9	4.1	3.7	4.4
"	Saline	4.1	6.0	4.9	5.0	3.7	5.1

As seen in Table VII, the heat-killed cells of *Esch. coli* exhibited the same activity as the endotoxin prepared from this organism with regard to both water intake and weight gain of the animal. In contrast, the staphylococcal cells were essentially inactive from both points of view. There was definite evidence of inhibition in the animals treated with heat-killed mycobacteria (BCG); but the activity of the mycobacterial cells was very much smaller than that of the Gram-negative bacilli. The inhibition of water intake and of weight gain by fractions of mycobacteria will be considered in greater detail in a subsequent publication.

8. Effect of Diet on Inhibition of Water Intake by Endotoxin.—In a series of tests that need not be reported in detail, it has been found that inhibition of water intake by endotoxin occurs whatever the diet fed the experimental animals. For example, Table VIII presents comparative results obtained with mice fed a complete diet (D & G) or a very inadequate diet (corn) for a period of 2 weeks before injection of endotoxin. Similar results have been obtained with mice fed for a period of 6 weeks diets containing as source of protein

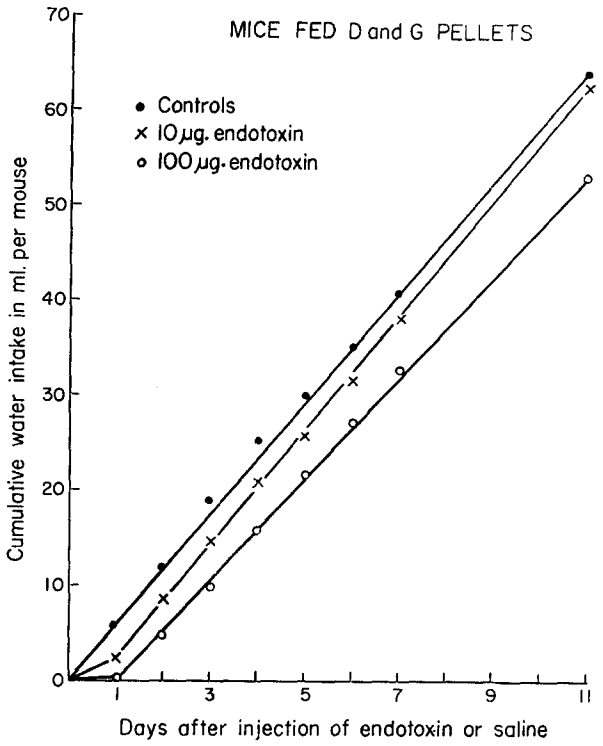

FIG. 3. Cumulative water intake of mice fed D & G diet and treated with 100 μg. or 10 μg. of endotoxin at time 0; controls received saline.

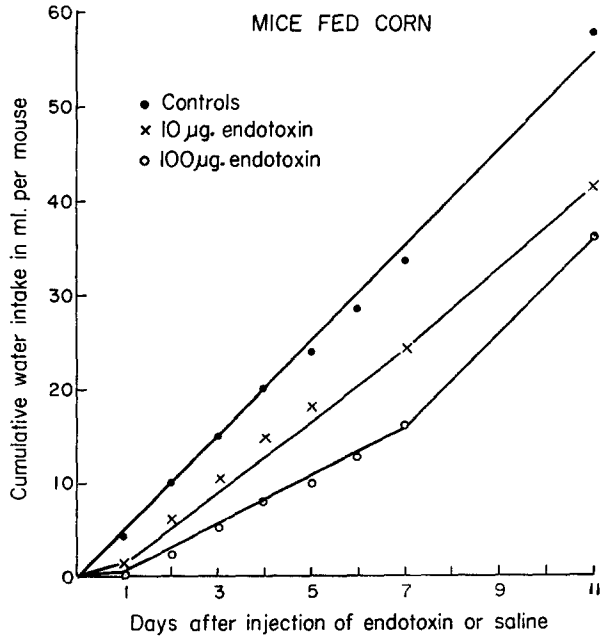

FIG. 4. Cumulative water intake of mice fed corn diet and treated with 100 μg. or 10 μg. of endotoxin at time 0; controls received saline.

either 15 per cent casein, or 15 per cent gluten, or 15 per cent gluten supplemented with lysine, threonine, and valine (5).

In the course of these experiments, it was observed that the time needed following injection of endotoxin to allow the daily water intake to return to a normal level was much longer in animals fed inadequate diets than in animals fed the D & G diet. The relation of dietary constituents to the length of time required for recovery from the endotoxin effect will be considered in another publication. One example will suffice to illustrate the phenomenon here.

Male mice 5 weeks old were fed either D & G diet or corn *ad lib.* After 2 weeks on these diets they received by the intraperitoneal route either saline or 100 or 10 μg. of endotoxin (prepared from *Esch. coli* 17). The daily water intake was measured as usual, the animals continuing to receive throughout the test the same diet they had received before treatment.

Figs. 3 and 4, corresponding respectively to the D & G diet and ground corn, present the cumulative daily water intake of the various groups of animals. The figures make clear that mice fed D & G diet recovered very rapidly from the effect of endotoxin, whereas the water intake was still markedly depressed 1 week after treatment in the animals fed corn.

SUMMARY

Injection of endotoxin of Gram-negative bacilli into NCS mice caused an immediate reduction or interruption of water intake by these animals, with a resultant loss of body weight.

Endotoxins prepared by three different techniques from four different cultures of Gram-negative bacilli yielded products having approximately the same activity in inhibiting water intake. The minimum effective dose was 0.1 μg. or less. With all toxin preparations tested, the duration of the effect was directly related to the dose injected.

The heat-killed cells of *Esch. coli* proved approximately as effective as the endotoxins prepared from Gram-negative bacilli. In contrast, heat-killed cells of *Mycobacterium tuberculosis* (BCG) were much less active, and heat-killed cells of *Staphylococcus aureus* were essentially inactive.

Mice previously treated with endotoxin exhibited a marked degree of tolerance to the inhibition of water intake caused in normal animals by a subsequent treatment with the same material. Tolerance could also be induced by vaccination with heat-killed Gram-negative bacilli. Tolerance overlapped from one bacterial species to another but was more pronounced toward the endotoxin prepared from the bacterial culture with which the animal was vaccinated.

The duration of the inhibitory effect of endotoxin on water intake was much shorter with mice fed a complete diet than with mice fed a deficient diet (corn).

It took approximately the same dose of endotoxin (0.1 μg.) to inhibit water intake, reduce the influx of polymorphonuclear leucocytes, and enhance staphylococcal infection.

BIBLIOGRAPHY

1. Dubos, R. J., and Schaedler, R. W., The effect of the intestinal flora on the growth rate of mice and on their susceptibility to experimental infections, *J. Exp. Med.*, 1960, **111,** 407.
2. Schaedler, R. W., and Dubos, R. J., The susceptibility of mice to bacterial endotoxins, *J. Exp. Med.*, 1961, **113,** 559.
3. Dubos, R. J., and Pierce, C. H., Differential characteristics *in vitro* and *in vivo* of several substrains of BCG. I. Multiplication and survival *in vitro*, *Am. Rev. Tuberc. and Pul. Dis.*, 1956, **74,** 655.
4. Smith, J. M., and Dubos, R. J., The behavior of virulent and avirulent staphylococci in the tissues of normal mice, *J. Exp. Med.*, 1956, **103,** 703.
5. Dubos, R. J., and Schaedler, R. W., Effect of nutrition on the resistance of mice to endotoxin and on the bactericidal power of their tissues, *J. Exp. Med.*, 1959, **110,** 935.
6. Berry, J., and Smythe, D. S., Effects of bacterial endotoxins on metabolism. III. Nitrogen excretion after ACTH as an assay by endotoxins, *J. Exp. Med.*, 1961, **113,** 83.
7. Cohn, Z. A., and Morse, S. I., Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxin, *J. Exp. Med.*, 1960, **111,** 689.
8. Fruhman, G. J., The mobilization of neutrophils, *Tr. New York Acad. Sc.* 1960, **23,** 30.