EFFECTS OF BACTERIAL ENDOTOXIN ON WATER INTAKE, FOOD INTAKE, AND BODY TEMPERATURE IN THE ALBINO RAT*

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In 1961, Dubos and Schaedler (1) reported a reduction in water intake in animals treated with bacterial endotoxin. Mice were raised in a pathogen-free environment and subsequently tested with one of several toxins. Alteration in daily water intake was found to occur at doses well below the LD_{50}. Because of the long-standing interest in this laboratory in drive states, their behavioral consequences, and physiological basis (2), this property of endotoxin was explored in the albino rat. Our animals were not raised in a pathogen-free environment. Using fairly large doses of toxin, we were able to confirm the findings of Dubos and Schaedler, and, in addition, demonstrate a profound toxin effect on food intake and body temperature. Using behavioral and physiological techniques, we made exploratory studies of resistance and sensitization to toxin and of its possible site of action.

Materials and Methods

Animals.—All the animals were male Sprague-Dawley albino rats, 90 to 120 days old, weighing 300 gm or more. They were housed in individual wire cages in a temperature-controlled room, and were fed Purina lab chow and tap water.

Water intakes were measured by making water bottles available for 30 minutes every day and weighing the bottles before and after consumption. After several "familiarization" days the rats would drink a fairly standard amount each day.

Food intake was measured by weighing the food in the cages every 24 hours, the food being continuously available. Animals on food-intake measurement were given water ad lib and those on water-intake measurement were given food ad lib.

Temperatures were taken with an ordinary rectal thermometer, lubricated and inserted almost its entire length. The animals showed no unusual distress and tolerated the procedure day after day. Thermometers were left in place 3 to 5 minutes and then read. Thermometers that dropped out were replaced for 3 additional minutes. A paper towel on the cage floor facilitated recovery of lost thermometers.

Toxin.—A commercially prepared lipopolysaccharide extract of *Escherichia coli* was used (Difco Laboratories 0880, Difco Laboratories, Detroit). The toxin was dissolved in saline and

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injected intraperitoneally in volumes not exceeding 0.4 cc of solution. Control injections of 0.4 to 0.5 cc of isotonic saline were used.

Behavioral Apparatus.—Fifteen animals were trained in a small sound-attenuated box where two metal levers were available. One was inactive, the other would deliver a small dipper of water (0.1 cc) or a pellet of food. Once the rat had learned this response, he was shifted to an automatically programmed schedule which delivered a reward for a bar press at irregular intervals averaging one reward every 30 seconds. On this schedule, the animal would press the bar at uniform rates for 1 hour each day. Responses were recorded on a counter and also on a cumulative recorder which plotted total responses against time and gave a convenient record of the rate of response. A small window in the box made direct observation possible.

Bacteriological Study.—A spot check of the stools of five of our animals showed them all to contain *E. coli*.[1] Since the animals were housed in group cages prior to the onset of the experiment and were all provided by the same commercial laboratory, we can assume that all our animals were infested with the organism.

RESULTS

Preliminary exploratory work suggested that an LD_{50} dose of *E. coli* endotoxin for our rats lay somewhere between 3 and 5 mg/kg and a dose that effectively reduced water intake somewhere near 0.75 mg/kg. A dose-response study of toxin doses below 0.75 mg/kg indicated that the relationship between the size of the dose and the amount of water drunk during a test period 2 hours later was linear. However, the number of animals used in this pilot study is too small (three to four in each dose group) to draw definite conclusions.

Effect on Water Intake.—0.75 mg/kg of endotoxin injected intraperitoneally would not affect water intake at once. Animals tested immediately after injection drank a normal amount. The time course of the toxin effect on drinking was studied by two methods. In the first experiment, groups of six animals were injected with 0.75 mg/kg of toxin at different time intervals before their usual drinking period. Fig. 1 shows the results of this experiment. The maximum reduction in water occurred at 2 hours. This intake was significantly different from control days ($P < 0.005$).

There was little observable effect of toxin on water intake at 48 hours. More recent studies[2] of licking rate in albino rats treated with endotoxin indicate an effect in the second 24 hours after injection. These observations have yet to be reconciled.

The second experiment involved the bar-pressing apparatus and will be described in detail below.

Effect on Food Intake and Temperature.—Daily food intake was measured in another group of six rats 24 and 48 hours after injection. There was a striking decrease on both days ($P < 0.005$ for 24 hours). Water was continuously avail-

[1] Dr. A. M. Jonas, Yale University School of Medicine, was kind enough to perform the bacterial culture studies for us.

[2] Stricker, E., and Miller, N. E., unpublished observations.

able to these animals but their fluid intake was also reduced by the toxin. Thirsty animals will refuse to eat dry food.

Two controls were used for this dehydration effect. Animals were deprived of water for 24 hours and then allowed to drink ad lib for 1 hour before injection. 24-hour food intake was measured beginning 3 hours after injection, water

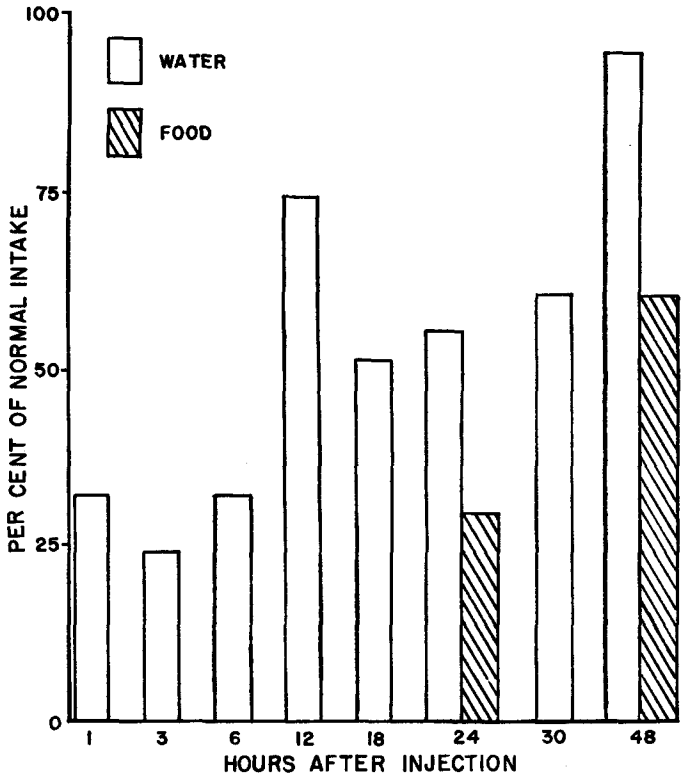

FIG. 1. Effects of toxin injection on food and water intake. Each bar represents the average intake of six rats.

being continuously available. Food intake fell to 30 per cent of normal for six animals tested.

In another series all six animals were anesthetized and stomach tubed after injection and three were given 10 cc of water by tube. Water was available throughout the test. Drop in food intake was dramatic for both groups, to 4 per cent of normal, but there was no difference between those force-watered and those not so treated.

Since the usual effect of bacterial toxins on body temperature is to produce fever, we were surprised to find a marked decrease in temperature in our animals. In a groups of eight rats the average drop in internal temperature was 3°F. A dose-response study of four doses of toxin and the drop in body temperature indicated a linear relationship between size of dose and decrease in temperature. Again, however, the number of animals studied is too small to be significant.

Repeated measurements were made after injection in two rats. The temperature started to fall by 30 minutes, reached a nadir at 2 hours, and returned gradually to normal by 6 to 8 hours. A repeat injection of 0.75 mg/kg of endotoxin 3 to 5 days later had no effect on body temperature in these animals.

The effect of typhoid vaccine (1 million killed organisms per cc) on temperature was tested in three animals and on water intake in an additional six animals. 1 cc injected intraperitoneally caused the body temperature to rise rapidly an average of 1.2°F and return to normal in 4 hours. Typhoid vaccine had no apparent effect on the amount of water drunk 1 hour after injection.

General Behavior and Variability.—Animals affected by the toxin usually lay quietly on the floor of their cages. They remained alert and would move spontaneously. Most of them would approach the water bottle and drink when it was first presented. They would often squeal loudly when picked up, but would not squirm or bite. Approximately half the animals would pass several watery foul-smelling stools during the first few hours after injection. An occasional rat would not drink at all for 24 to 48 hours and still survive. Sometimes an animal would show no change in water intake following injection. Of the forty-two rats used in the experiment illustrated in Fig. 1, five had normal water intakes despite toxin injection.

Bar Pressing Study.—Fifteen rats were trained to press a lever at a steady rate for an intermittent food or water reward. The cumulative recorder records of a typical animal are shown in Fig. 2. Animals were tested following 24 hours of deprivation.

When the animals were producing reliably similar rates of bar pressing daily, they were tested after intraperitoneal injections of normal saline or 0.75 mg of toxin. The saline injections did not change the bar pressing rate.

The effect of toxin injection can be seen in Fig. 2. Immediately after injection, the rate remains normal, but at 30 to 40 minutes following injection, the record flattens out, indicating few if any responses for the rest of the hour. Put back into their home cages, the rats will drink 10 cc of water from their usual water bottles. This effect was seen in eight animals tested with water reward and three animals with food reward. In three animals tested with water there was no effect on bar pressing rate, and one rat showed an immediate decrease in rate of response but did not stop completely.

The cumulative recorder records give an accurate and dramatic illustration

of the suddenness and time of onset of the toxin effect. It would be difficult to obtain this data from measurements of intake alone. The value of using several methods to measure changes in drive state has been previously demonstrated in the study of the effect of brain lesions on food intake (3).

Effects of Repeated Injections.—The frequent occurrence of resistance to

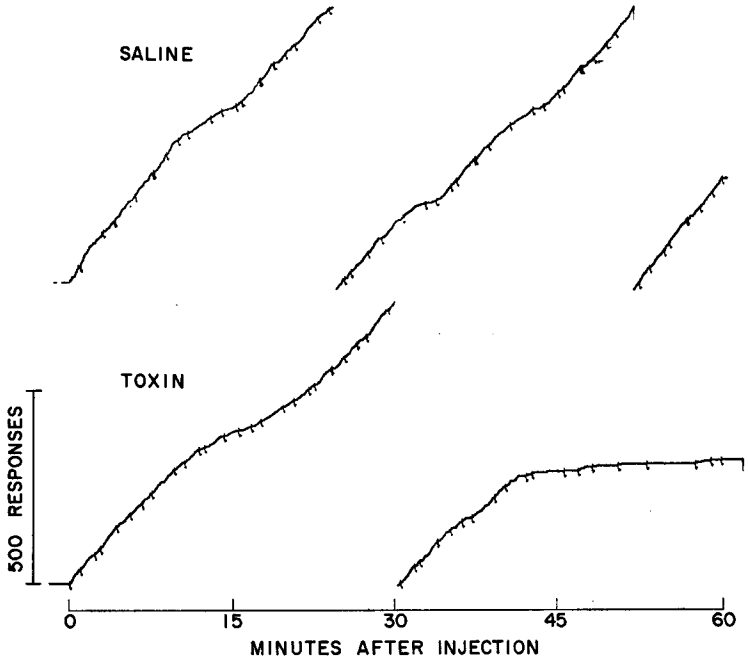

FIG. 2. Cumulative records of bar-pressing responses in a single animal. Each response on the bar raises the pen a small increment. At the upper limit of its excursion the pen automatically resets to zero. Each small downstroke of the pen marks the arrival of a dipper of water. Upper record shows 1-hour run following intraperitoneal saline injection. Lower record from next day shows the effect of toxin injection (0.75 mg per kg). At 40 minutes the response rate drops to nearly zero.

toxin, as well as of an occasional animal apparently extremely sensitive to it, raised the question of the effect of repeated injections. Preliminary exploration, as well as the literature on the febrile effects of toxins (4), suggested that animals should be resistant to toxin up to 5 days after injection and resusceptible after 10 to 15 days.

Eight naive rats were adapted to the 30 minute watering followed by rectal thermometer routine. They were then injected with toxin 2 hours before testing on three separate occasions, at 7, 11, and 26 days. The dose used was always 0.75 mg/kg. Fig. 3 shows the results for the entire group. The response on day 7

appears to be somewhat less than on the other 2 days. The drop in water intake and temperature on day 11, however, is largely due to two animals who had shown no response to the first injection.

More dramatic results were obtained with repeated toxin injections in animals trained in the bar-pressing box. Data from seven of these animals is summarized in Table I. There appears to be a period up to 10 days after injection in which the animal may show little effect of a repeat injection of the same dose of toxin. In three cases, however, a repeat injection 14 to 32 days after the first produced

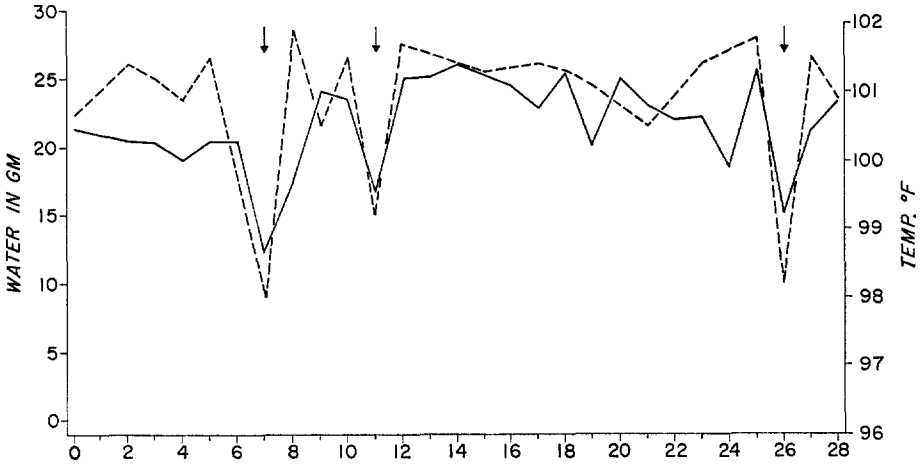

FIG. 3. Water intake (solid line) and rectal temperature (broken line) in eight rats subjected to repeated injections of toxin (arrows) on days 7, 11, and 26.

an immediate and severe depression of response rate. In two animals the effect of a second injection was the same as that of the first. Fig. 4 shows the records of an animal demonstrating both "protection" and "sensitization" effects.

Effects of Intracranial Injection of Toxin.—It has been previously demonstrated that electrical stimulation in the hypothalamus may produce eating or drinking (5). In addition, Grossman (6) has shown similar effects from stimulation achieved by the introduction of minute amounts of solid chemicals through a cannula permanently implanted in the animal's brain. We studied six rats using Grossman's technique. Twenty-two gauge hypodermic tubing with an attached stylet was implanted in one side of the rat hypothalamus and an insulated steel needle, bared at the tip, on the other side.[3] The animals were trained to bar press for water on the variable reward schedule used in the previous experiments. A small lesion was then made in one side of the hypothalamus by passing a 1.5 microampere DC current for 15 seconds between the needle

[3] Miss Elizabeth Sherwood performed the stereotaxic surgery on these animals.

electrode and a rectal probe.[4] All animals recovered from the lesion but one showed a much slower and more variable rate of bar pressing even 1 month afterwards and was discarded. The other five rats showed no change in bar pressing rate after the lesion. Subsequent histological examination revealed ex-

TABLE I

Effect of Repeated Toxin Injections on Rate of Bar Pressing for a Water Reward

Type of reaction	Days since previous injection	No. of Cases
Normal: Response rate depressed at 30 to 40 min...	8 and 15	2
Protected: Little or no effect.....	4 to 10 inclusive	7
Sensitized: Immediate marked reduction in response rate.....	14, 17, and 34	3

N = 7.

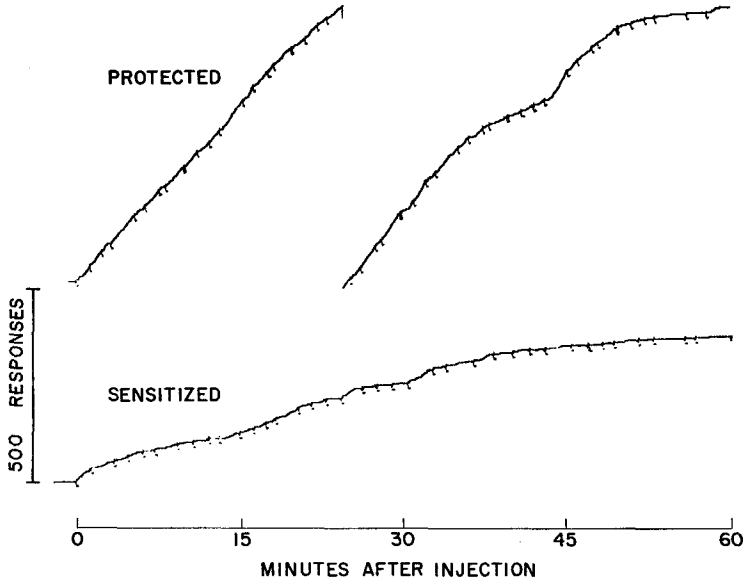

FIG. 4. Bar-pressing records from same animal shown in Fig. 2. On both days the animal was injected with toxin just before testing. Upper record, 5 days after first injection. Lower record, 14 days later. Control records on intervening days remained normal.

tensive hypothalamic lesions involving much of the lateral area between the levels of the optic chiasm and the mammillary body. The mammillary nucleus itself was not injured.

The rationale of the lesions was to limit activity of the hypothalamic mecha-

[4] The lesion making equipment was loaned to us by Dr. Frederick Gault.

nisms to one side. Failure of injections into the unlesioned area to alter behavior could then not be assumed to be due to continued function of the opposite side. Each of the five animals was run for 1 hour in the bar-pressing box immediately following the injection through the hypothalamic cannula of 0.0025 mg of toxin dissolved in 0.1 microliter of saline. Since the animals weighed slightly over 300 gm, this gave a dose of 0.007 mg/kg. From the dose-response study mentioned earlier, it was predicted that this dose might have a slight effect on water intake. The bar-pressing test was used because we were interested in the time of onset of any effect and because of our conviction that it was a more subtle indicator of toxin action than water intake.

If the implanted cannula did indeed lie in the area of the brain constituting a site of action of the toxin, the time of action might be more rapid and the effect greater because of the concentration of toxin at its active site. Control injections of 0.1 microliter of saline and of 0.1 mg of lidocaine (xylocaine ®, a local anesthetic) dissolved in 0.1 microliter of saline were made.

Four of the injection cannulae lay in the lateral hypothalamic area. The tip of the other lay outside the hypothalamus above the optic tract.

Saline injections had no effect on bar-pressing rate. Toxin injection slowed the response rate slightly in two animals, one of which was the rat with the misplaced cannula.

The effects of the lidocaine injections were dramatic. Bar pressing stopped almost immediately after the animals were placed in the apparatus. The rats lay quietly on the floor of the cage, but could be aroused by handling or by tapping on the box. After a period of 10 to 30 minutes they resumed bar pressing at a normal rate. One animal showed no effect from lidocaine injection, but this was the animal in which the cannula lay outside the hypothalamus.

We felt these controls were adequate to assure us that the cannulae were patent and lay in areas where behavioral effects could be produced by an active substance.

Since the toxin itself was ineffective, we sought some other factor which might act on the hypothalamus.

Atkins and Wood (10) have demonstrated an endogenous pyrogenic factor in the sera of rabbits treated with toxin. Because some such intermediary substance, rather than toxin itself, might be involved in the water intake suppression, we undertook to test such sera in the animals with hypothalamic cannulae. Blood was drawn from two rats by cardiac puncture two hours after toxin injection. Both animals were obviously ill, and had diarrhea. The blood was allowed to clot, centrifuged, and the sera drawn off, combined, and stored under refrigeration. Injections of 0.1 to 0.2 microliters of this substance through the cannulae in four animals had no effect on their bar-pressing rate. In comparison with the large amounts of serum used to produce fever in rabbits, however, these doses may be below any possible response level. Other explanations

include the possibilities that our sera did not contain any active factor, or that it was not being injected into a receptive site in the brain. There are reasons to believe that a better site in the posterior hypothalamus could be found.

DISCUSSION

The effects of bacterial endotoxin on water intake reported by Dubos and Schaedler (1) and on food intake reported by Berry (7) are similar to those obtained in our rats. Berry found that in toxin-treated mice the food did not pass the upper intestine. The frequent occurrence of diarrhea indicates that a different mechanism may have been active in our animals.

While bacterial toxins have been reported to cause a fall in temperature in some species of animals, Feldman and Gellhorn (8) found a rise in temperature with typhoid vaccine in the white rat. We replicated this effect with typhoid vaccine as a control study. The typhoid vaccine had no effect on water intake in these animals. We observed only a depression of body temperature by *E. coli* endotoxin even with doses of 0.01 mg/kg. This suggests that the mechanism of action of *E. coli* endotoxin is qualitatively different from that of typhoid toxin.

The marked individual variation in response to endotoxin in our animals has been observed by others. Dubos and Schaedler found changes in susceptibility to the lethal effect of toxin with exposure to the *E. coli* organism. We assume, on the basis of stool examination in five animals, that all our rats were exposed and infested with *E. coli*, but this factor was not controlled.

The site of action of the endotoxin is unknown. We assumed it likely to be the hypothalamus, but our hypothesis is certainly not supported by our inability to produce any change in the sensitive bar-pressing test by injecting toxin into one side of the hypothalamus.

The behavioral testing enabled us to time the exact onset of the toxin effect. This led to the discovery of a hypersusceptible state in some of our animals 14 to 30 days following a toxin injection. These "sensitized" animals would show an immediate decrease in response rate following injection, instead of the usual reaction after 30 minutes. We feel that the information to be gained from the use of several behavioral measures rather than a single one (such as volume of water intake) justifies the additional work required.

The data presented here indicate that toxin depresses all on-going activity: eating, drinking, working on bar-pressing apparatus. We have preliminary data, however, that this is not always the case. Rats in a rotating drum can be taught to press a lever to stop the rotation for brief periods (9). Under these circumstances endotoxin injection seems to increase the rate of response (unpublished observations). Further study of toxin effects on such complex learned behavior may shed some light on the phenomena of "feeling sick."

SUMMARY

Intraperitoneal injections of *Escherichia coli* endotoxin in albino rats produces a decrease in food and water intake and a drop in body temperature.

The drop in temperature and in water intake is probably proportional to the size of the dose.

Using a behavioral test in which animals are trained to press a bar at a steady rate for intermittent food or water reward, it is possible to demonstrate the sudden onset of the toxin effect at 30 to 45 minutes after injection.

In any group of rats, all of whom were presumably exposed to *E. coli*, three types of response to toxin can be found: (*a*) Sharp reduction in water intake 30 minutes after injection. (*b*) Little or no change in intake or rate of working for water reward. (*c*) Immediate depression of work rate.

These three types of reaction appear related to previous experience with the toxin. The "normal" or "inexperienced" reaction *a* was seen in animals who had not been given toxin before. The "protected" reaction *b*, with little or no effect of toxin injection on response rate was frequently found 4 to 5 days after a previous injection. The "susceptible" reaction *c* was found in three animals after 14 or more days had passed since a previous injection.

Injections of toxin into the lateral hypothalamic region of four animals through implanted cannulae had no effect on the rate of bar pressing for water. Control injections of lidocaine blocked response rate completely for brief periods in three animals.

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