Effects of Endotoxin on Monoamine Metabolism in the Rat (36543)

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Bacterial endotoxins enter the circulation continuously from the gut in the normal state and undergo rapid detoxification within the reticuloendothelial system (1). Small amounts of circulating endotoxin evoke a transient fever, but larger amounts evoke sympathetic hyperactivity, a fall in body temperature and functional disturbances that result from a generalized increase in membrane permeability. The primary injury is to the peripheral circulation which displays progressive hemodynamic deterioration, a decline in venous return until cardiac output falls to a level incompatible with survival.

The systems involved in regulation of both body temperature (2) and blood pressure (3) are thought to include central monoaminergic neurons, peripheral sympathetic neurons and the adrenal medulla. Since endotoxin has been shown to release at least one putative neurotransmitter [GABA; ref. (4)] and since intraventricular endotoxin rapidly evokes vascular collapse (5), we have examined the effects of endotoxin administered intraperitoneally and intracisternally on monoamine metabolism in brain and selected peripheral tissues.

Materials and Methods. Female Charles River Sprague-Dawley rats weighing 200–250 g were housed 3 per cage in a room maintained at 20° and illuminated from 6 a.m. to 6 p.m. with Vita-Lite (Duro-Test Manufacturing Co., North Bergen, NJ). Purina chow and water were available *ad libitum*. Animals were decapitated and their tissues were frozen on dry ice and stored at −20° until assayed. In some experiments, the brain was dissected into two parts, denoted as "brainstem" and "telencephalon," by making a transverse cut at the level of the optic chiasm, just posterior to the anterior commissure. The part of the telencephalon remaining attached to the brainstem was separated and pooled with the rostral areas of the brain. *dl*-7-^{3}H-Norepinephrine (10.8 Ci/mmole) was obtained from the New England Nuclear Corporation, Boston, MA. Endotoxin was dissolved in buffered saline and injected intraperitoneally or intracisternally. ^{3}H-Norepinephrine was injected into the tail vein (35 μCi in 0.2 ml), or intracisternally (6 μCi in 0.02 ml), after the pH had been adjusted to 6.5. For the intracisternal injections, the animals were lightly anesthetized with ether (6). Body temperature (°F) was measured with a Yellow Springs rectal probe (Yellow Springs Instrument Company, Yellow Springs, OH). Animals were kept at an ambient temperature of 20° during these data readings. All experimental groups contained 8 or 9 animals.

Estimation of ^{3}H-norepinephrine and its metabolites. Tissues were homogenized in 5 vol of ice-cold 0.4 *N* perchloric acid and centrifuged at 17,000*g* for 20 min. The supernatant fluid was passed over alumina columns at pH 8.6 (7). Unchanged ^{3}H-norepinephrine in each sample was determined by measuring radioactivity in an aliquot of the acetic acid eluate from the alumina columns (8). To assay the metabolites of ^{3}H-norepinephrine, each alumina effluent was pooled with the first 5 ml of the buffered acetate solution used to wash the alumina columns, and passed over an ion exchange column (Dowex 50W-X4, 200-400 mesh, H^+

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form) (9). Tritiated, *O*-methylated, deaminated metabolites were collected in the effluents from these columns and the ^{3}H-normetanephrine was then eluted with 3 *N* NH_4OH (9).

Estimation of endogenous amines. Portions of the acetic acid eluate of the alumina columns were used to estimate endogenous catecholamine levels by the method of von Euler and Lishajko (10). Tissues were homogenized in acidified butanol and the serotonin was determined by the *O*-phthaldialdehyde method of Maickel *et al.* (11).

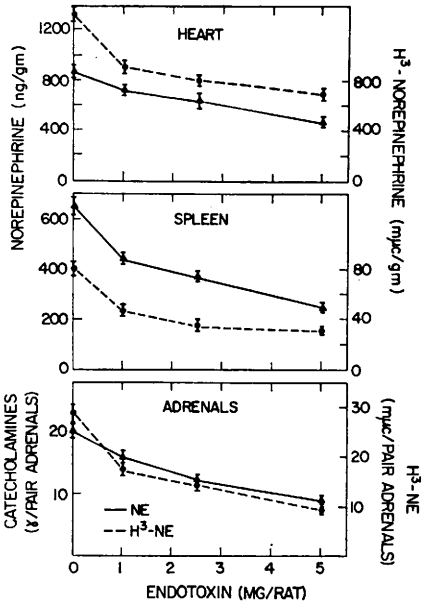

FIG. 1. Dose response of the effect of endotoxin on peripheral catecholamine content. Rats received 35 μc of ^{3}H-norepinephrine intravenously 1 hour before the intraperitoneal administration of endotoxin.

Results. Effect of endotoxin on tissue catecholamine metabolism. 1. Endogenous catecholamine levels. Intraperitoneal administration of endotoxin caused the depletion of endogenous catecholamines in all three peripheral organs examined (*i.e.*, heart, spleen, and adrenals) (Fig. 1). Animals were injected with varying doses and killed 5 hr later. The highest dose of endotoxin tested (5 mg) produced about a 50% decline in the concentrations of endogenous catecholamines in the heart, spleen, and adrenals. Significant depletions were observed with even the lowest dose tested (1 mg). Some of the animals that received 5 mg developed diarrhea; on autopsy, prominent hemorrhagic lesions were observed in the lungs of some of these animals as would be characteristic of a lethal dose. Administered endotoxin also depleted brain norepinephrine, either when injected

TABLE I. Effects of Endotoxin Administration on Brain Norepinephrine Concentration.[a]

Endotoxin dose (mg/rat)		Norepinphrine (ng/g)
ic	ip	
0	—	421 ± 21
0.02	—	393 ± 20
0.05	—	365 ± 25[b]
0.10	—	336 ± 15[c]
0.20	—	300 ± 12[c]
—	0	457 ± 30
—	1.00	434 ± 22
—	2.50	401 ± 17[b]
—	5.00	325 ± 15[c]

[a] Animals were killed 5 hr after receiving endotoxin intracisternally (ic) or intraperitoneally (ip).

Differs from controls: [b] $p < .05$; [c] $p < .001$.

TABLE II. Effect of Intraperitoneal Endotoxin on Brain Norepinephrine and Serotonin Concentrations.[a]

	Saline	Endotoxin
Brainstem		
Norepinephrine (ng/g)	502 ± 9	477 ± 4[b]
^{3}H-Norepinephrine (nCi/g)	289 ± 25	222 ± 15[b]
Serotonin (ng/g)	642 ± 18	687 ± 33
Telencephalon		
Norepinephrine (ng/g)	219 ± 13	180 ± 5[b]
^{3}H-Norepinephrine (nCi/g)	61 ± 5	33 ± 4[c]
Serotonin (ng/g)	355 ± 16	352 ± 7

[a] Animals were injected with endotoxin (5 mg ip) 1 hr after the intracisternal injection of ^{3}H-norepinephrine; they were killed 5 hr later.

Differs from controls: [b] $p < .05$; [c] $p < .001$.

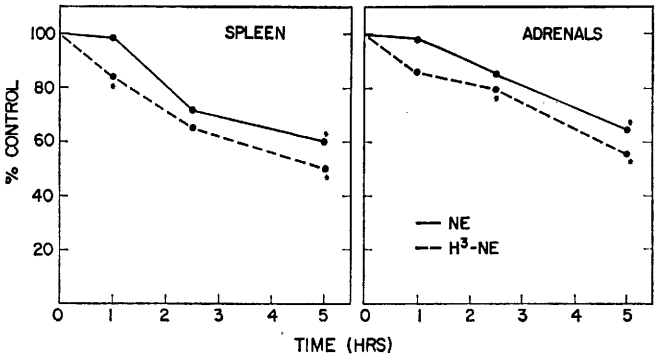

FIG. 2. Time course of the effect of endotoxin on catecholamine content. Animals received 4 mg of endotoxin intraperitoneally 1 hr after the intravenous administration of ^{3}H-norepinephrine. They were killed after various intervals, as indicated ($*p < .05$ differs from saline-treated animals).

peripherally (2.5–5.0 mg/rat) or directly into the cerebrospinal fluid (50–200 μg/rat) (Table I).

Brain serotonin levels were not altered 5 hr after rats had received a 5 mg dose of endotoxin ip (Table II).

2. Disappearance of ^{3}H-catecholamine. Exogenous endotoxin (1 mg) accelerated the disappearance of ^{3}H-catecholamine from the heart, spleen, and adrenals (Fig. 1). Animals received ^{3}H-norepinephrine intravenously 1 hr before the intraperitoneal endotoxin. This acceleration of ^{3}H-norepinephrine disappearance was a more sensitive indicator of the effects of endotoxin on catecholamine storage than the depletion of endogenous catecholamines (*i.e.*, the effects of the 1-mg dose on ^{3}H-norepinephrine disappearance were greater) (Fig. 1).

The time course of the depletion of splenic and adrenal catecholamines was examined in animals injected with intravenous ^{3}H-norepinephrine, and with intraperitoneal endotoxin (4 mg) 1 hr after the amine administration, and killed 1, 2.5, or 5 hr after injection of endotoxin. One hour after endotoxin administration, ^{3}H-norepinephrine content had decreased 14% in the adrenals and 16% ($p < .05$) in the spleen. The fall was more pronounced by 2.5 hr; by 5 hr, ^{3}H-norepinephrine levels were 45% lower in the adrenals, and 50% lower in the spleen than in those of control animals (Fig. 2). The depletion of endogenous catecholamines by en-

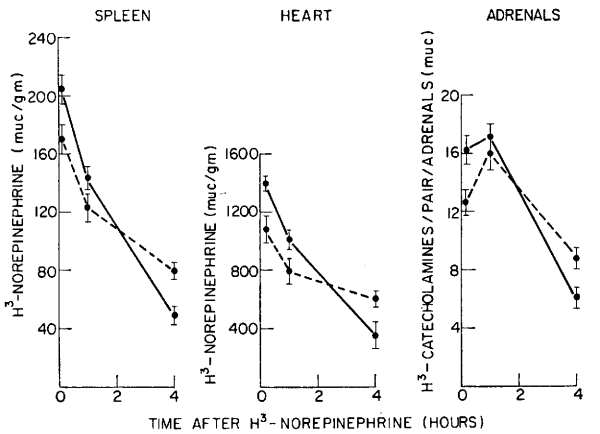

FIG. 3. Effect of endotoxin on ^{3}H-norepinephrine uptake. Animals received ^{3}H-norepinephrine intravenously 1 hr after intraperitoneal endotoxin. They were killed at the time intervals indicated after ^{3}H-norepinephrine administration. (—) endotoxin-treated rats; (- -) control animals.

dotoxin was less marked than that of ^{3}H-norepinephrine: One hour after endotoxin administration, catecholamine levels in both the adrenals and the spleen fell by only 2%. Five hours after endotoxin, the declines in endogenous catecholamine levels were significant, but were of lesser magnitude (by about 10%), than the decline in labeled catecholamines.

A radioisotopic label was introduced into the brain catecholamine stores by injecting ^{3}H-norepinephrine intracisternally. The subsequent intraperitoneal administration of endotoxin (5 mg) caused a significant acceleration in the disappearance of the ^{3}H-norepinephrine from the brainstem and the telencephalon (Table II).

3. Uptake and metabolism of ^{3}H-norepinephrine. Pretreatment of rats with intraperitoneal endotoxin (4 mg) increased the proportions of administered ^{3}H-norepinephrine that were taken up by the heart, spleen, and adrenals. The ^{3}H-norepinephrine was injected intravenously 1 hr after the endotoxin; the animals were killed at various intervals thereafter. The uptake of ^{3}H-norepinephrine 10 min after its intravenous administration was significantly increased in the heart, spleen, and adrenals of these animals (Fig. 3). Differences between the retention of ^{3}H-norepinephrine in the examined tissues of endotoxin-treated and control rats were no longer apparent after 1 hr. By 5 hr, the ^{3}H-norepinephrine contents of these organs were *lower* in the endotoxin-treated rats, which suggests that the toxin both increased the uptake and accelerated the turnover of the catecholamine.

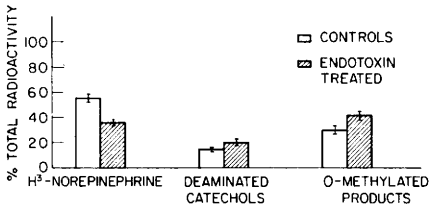

FIG. 4. Effect of endotoxin on ^{3}H-norepinephrine metabolites in rat brain. Animals received 2.5 mg of endotoxin intraperitoneally 1 hr after the intracisternal administration of ^{3}H-norepinephrine. They were killed 1 hr later. Bars represent standard errors of the mean.

To examine the metabolic form in which endotoxin liberates norepinephrine from central neurons, we identified and measured the major metabolites of ^{3}H-norepinephrine present in the brain 4 hr after intraperitoneal endotoxin, and 5 hr after the intracisternal administration of ^{3}H-norepinephrine. Brains of endotoxin-treated rats contained 19% less ^{3}H-norepinephrine ($p < .001$), and 10% more of the deaminated ^{3}H-metabolites ($p < .05$) than those of control animals (Fig. 4). They also contained higher levels of the *O*-methylated metabolites of ^{3}H-norepinephrine.

Effect of endotoxin on rectal temperature. Both endotoxin and its diluent caused a

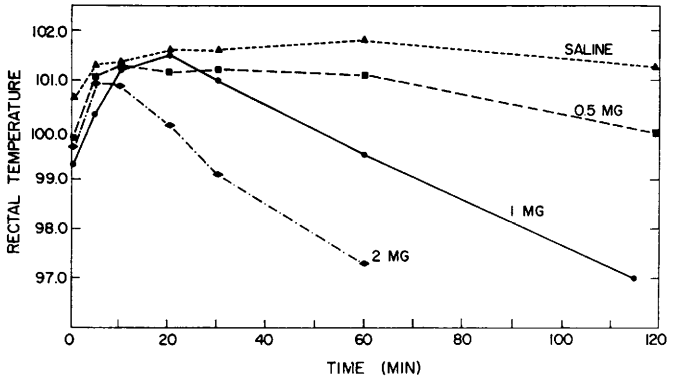

FIG. 5. Dose response of the effect of endotoxin on rectal temperature of the rat. Animals were injected intraperitoneally with various doses of endotoxin. Rectal temperature was measured at 10, 20, 30, 60 and 120 min after endotoxin administration.

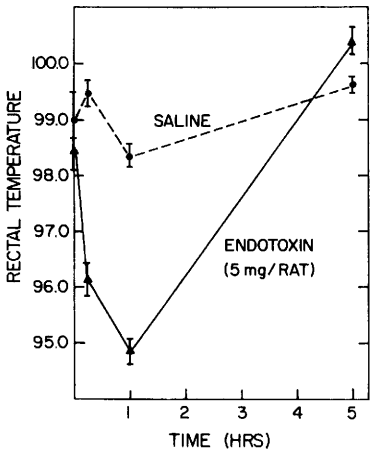

FIG. 6. Effect of endotoxin on the rectal temperature (mean values) of the rat. Animals received 5 mg of endotoxin intraperitoneally. Rectal temperature was measured at various times thereafter.

slight initial rise in rectal temperature (Fig. 5). In doses of 1 mg ip or greater, endotoxin caused a subsequent temperature decline which was apparent after 30 min in the animals that received 1 mg, and after 20 min in those that received 2 mg (Fig. 5). The administration of a 5-mg dose caused no initial rise in temperature, a peak hypothermic effect after 1 hr, and significant hyperthermia after 5 hr (Fig. 6).

Since the central administration of norepinephrine is known to alter body temperature (12), we examined the effect of the dose of ^{3}H-norepinephrine (0.03 μg) used to label central noradrenergic neurons. Neither this dose nor our usual intravenous dose (0.24 μg) had any effect on rectal temperature (Table III). The injection of ^{3}H-norepinephrine intracisternally or intravenously also did not modify the levels of endogenous norepinephrine in brainstem, telencephalon, spleen, heart or the levels of endogenous catecholamines in the adrenal medulla.

Correlation of catecholamine changes with changes in body temperature. To determine whether the changes in rectal temperature induced by endotoxin preceded or followed its effects on catecholamine metabolism, animals were given endotoxin (2 mg) 1 hr after they received both intracisternal and intravenous ^{3}H-norepinephrine. The rectal temperatures were recorded every 10 min for a period of 2 hr after endotoxin administration. After 50 min, rectal temperature decreased significantly when compared with that of saline-injected rats; however, by 2 hr, the temperature was slightly above that of controls (Fig. 7). Adrenal catecholamine levels were 10% lower 2 hr after endotoxin treatment; adrenal ^{3}H-catecholamine content was depressed by 17% ($p < .05$) after 20 min, and by 38% ($p < .05$) after 2 hr (Fig. 7). A similar pattern of norepinephrine and ^{3}H-norepinephrine depletion was observed in the heart and the spleen; splenic norepinephrine content first became significantly depressed (20%, $p < .05$) 30 min after endotoxin treatment.

TABLE III. Effects of ^{3}H-Norepinephrine Injection on Rectal Temperature and Tissue Catecholamine Concentrations.[a]

	Saline	^{3}H-Norepinephrine
Brainstem (ng/g)	656 ± 18	652 ± 13
Telecephalon (ng/g)	221 ± 26	234 ± 25
Spleen (ng/g)	859 ± 113	1126 ± 143
Heart (ng/g)	1588 ± 104	1500 ± 116
Adrenals (μg/pair)	18 ± 1	18 ± 1
Temp (°)		
control	97.7 ± 0.3	98.2 ± 0.3
1 hr	98.5 ± 0.7	99.3 ± 0.6

[a] Rats were briefly anesthetized with ether; ^{3}H-norepinephrine or saline was injected intravenously and intracisternally. Rectal temperature, measured every 10 min, failed to change significantly in either group. The animals were killed after 1 hr.

Endotoxin administration also accelerated the disappearance of ^{3}H-norepinephrine from the brain before it caused changes in the rectal temperature (Fig. 8): ^{3}H-norepinephrine content was significantly depressed in the brainstem after 20 min, and in the telencephalon after 30 min. This dose

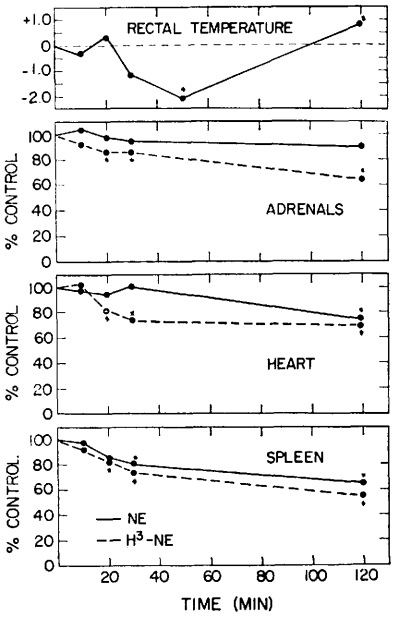

FIG. 7. Correlation of the effect of endotoxin on rectal temperature and peripheral catecholamines. Animals received 2 mg of endotoxin intraperitoneally 1 hr after labeling both central and peripheral catecholamines with ^{3}H-norepinephrine. The rectal temperature was measured at 10, 20, 30, and 120 min, at which time the animals were killed ($*p < .01$ differs from saline-injected controls). The top box represents the change in rectal temperature (°F) from control and the three lower boxes show the percentage of control values of endogenous and ^{3}H-norepinephrine obtained in animals given endotoxin and its diluent.

of endotoxin did not significantly alter levels of endogenous norepinephrine at either 20 or 30 min or in either brain region (Fig. 8).

Discussion. These studies indicate that endotoxin, administered intraperitoneally, lowers the norepinephrine content in peripheral sympathetic neurons (Figs. 1 and 2) and brain (Tables I and II), and the catecholamines in the adrenal medulla (Figs. 1 and 2); it also accelerates the disappearance of ^{3}H-norepinephrine from all these tissues (Fig. 2, Table II). Depletion of endogenous peripheral catecholamines during endotoxin shock has previously been noted in dogs (13). In addition to altering the levels of catecholamines in various tissues and the turnover of ^{3}H-norepinephrine, endotoxin treatment also enhances the uptake of circulating ^{3}H-norepinephrine into sympathetic nerve endings (Fig. 3), and modifies the metabolism of ^{3}H-norepinephrine in brain neurons; that is, it increases the proportion of intracisternally injected ^{3}H-norepinephrine recovered in the brain as *O*-methylated metabolites (Fig. 4). Endotoxin may, therefore, have direct effects on catecholamine storage mechanisms, perhaps similar to those responsible for the reduction of GABA that it produces in crayfish (4). Alternatively, the effects of endotoxin on peripheral catecholamine turnover could result from an increase in sympathetic tonus, a hypothesis compatible with the observation that if the spleen was acutely denervated prior to endotoxin administration, the endotoxin would no longer depress splenic norepinephrine content (14). Other mechanisms that could participate in catecholamine depletion by endotoxin

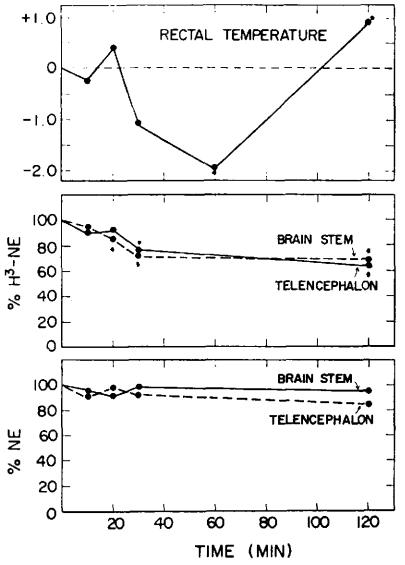

FIG. 8. Correlation of the effect of endotoxin on rectal temperature and on brain norepinephrine. Animals received 2 mg of endotoxin intraperitoneally 1 hr after labeling the administration of ^{3}H-norepinephrine intracisternally and intravenously. The rectal temperature was measured at 10, 20, 30, and 120 min, at which time the animals were killed ($*p < .01$ differs from saline-injected controls). The top box represents the change in rectal temperature (°F) from control and the three lower boxes show the percentage of control values of endogenous and ^{3}H-norepinephrine obtained in animals given endotoxin and its diluent.

include a suppression of catecholamine synthesis, a reserpine-like effect on its storage vesicles, and interference with its reuptake into noradrenergic neurons. The mechanism by which endotoxin *increased* the uptake of circulating ^{3}H-norepinephrine into sympathetic neurons within peripheral tissues (Fig. 3) remains obscure, but may be related to the acceleration that it produces in catecholamine turnover. A similar increase in ^{3}H-norepinephrine uptake was observed in peripheral sympathetic neurons that were stimulated electrically (15).

Both intraperitoneal and intracisternal injections of endotoxin caused significant depletion of norepinephrine and acceleration in turnover of ^{3}H-norepinephrine in brain (Tables I and II). We have not been able to locate data on the passage of endotoxin from the blood into brain in the rat. If we assume that the blood–brain barrier for the toxin is absent or incomplete, its effect on metabolism of brain norepinephrine could be attributed either to a direct effect on catecholamine-containing neurons or to reflex activation of these neurons, perhaps as a result of hypotension (3). Reflex activation alone would probably not cause a reduction of catecholamines. The increase in *O*-methylated metabolites of ^{3}H-norepinephrine within brains of endotoxin-treated rats suggests that at least part of the norepinephrine liberated after the administration of the toxin leaves brain neurons in a physiologically active form.

The effect of endotoxin on body temperature was found to be dose-dependent (Fig. 5). Low doses of the toxin produced only hypothermia; however, in rats given 5 mg, the hypothermia was followed by hyperthermia.

The mechanism by which pyrogens induce fever is unknown. Electrophysiological evidence suggests that they alter the sensitivity and neuronal activity of the thermoregulatory preoptic–anterior hypothalamic neurons (16, 17). Thus the sensitivity and firing rates of neurons sensitive to warmth was decreased, while that of neurons sensitive to cold was increased, which led to a decrease in heat loss and increase in heat production (16). The effects of iontophoretically applied norepinephrine are strikingly similar: There is an increase in firing of neurons sensitive to warmth, and a decreased firing rate of neurons sensitive to cold (18). Thus, it is possible that changes in body temperature induced by endotoxin are mediated through central noradrenergic neurons. In support of this hypothesis, we found that an increase in turnover of ^{3}H-norepinephrine in the brain preceded the changes in rectal temperature in endotoxin-treated animals (Figs. 7 and 8). Whether endotoxin acts directly on norepinephrine storage within nerve terminals is still uncertain. The delay in the changes in ^{3}H-norepinephrine turnover, in rectal temperature, and in the electrophysiological responses (16) might indicate that endotoxin acts first on peripheral or on other central neuronal systems.

Brain monoamines have been implicated in the control of body temperature. Feldberg and Myers (12, 19) have suggested that both norepinephrine and serotonin are involved. More recently, an increase in the turnover of hypothalamic ^{3}H-norepinephrine was found when animals were exposed to either low or high temperatures (20, 21). An increase in brain serotonin was found only when they were exposed to high temperatures (20, 22). In the rat, centrally administered norepinephrine produced a dose-dependent temperature response: low doses produced hypothermia, while high ones produced hyperthermia (23). Similarly, we found that endotoxin administration to rats resulted in profound hypothermia; in surviving animals that received higher doses (5 mg), this was sometimes followed by hyperthermia (Fig. 6).

If the acceleration of brain norepinephrine metabolism by endotoxin is a necessary component of its effects on body temperature, it might be possible to modify the effects of endotoxin on temperature regulation or on other visceral control mechanisms by treating animals with drugs that would block endotoxin effects on noradrenergic neurons in the brain.

Summary. We have examined the effects of administered endotoxin on catecholamine metabolism in rat brain, sympathetic neurons, and adrenal medulla. Intraperitoneal

endotoxin (1–5 mg) produced a dose-dependent depletion of splenic and cardiac norepinephrine and of adrenal epinephrine; it also accelerated the disappearance of ^{3}H-norepinephrine taken up from circulation. Pretreatment of animals with intraperitoneal endotoxin increased initial uptake of circulating ^{3}H-norepinephrine into heart and spleen. Endotoxin given intraperitoneally (2.5–5 mg) or intracisternally (50–200 μg) depleted brain norepinephrine; it accelerated ^{3}H-norepinephrine turnover in the rat brains in which norepinephrine had been radioisotopically labeled by intracisternal injection. Brain serotonin content was not affected by endotoxin. The acceleration of ^{3}H-norepinephrine turnover in brainstem and hypothalamus after endotoxin administration preceded the induction of hypothermia. This suggests that the effects of endotoxin on body temperature may be mediated in part by central noradrenergic neurons.

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