Some Effects of Endotoxin and Leukocytic Pyrogen on the Body Temperature of Rats (33996)

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Several investigators have shown that the rat, unlike some other species, responds to endotoxin injection with hypothermia (1–3). In the species that do respond to endotoxin with fever, an endogenous pyrogen can be demonstrated (4). It now appears likely that endotoxin may cause fever indirectly by liberating an intermediate endogenous pyrogen from the tissue cells of the host (5). Leukocytic pyrogen is a low molecular weight basic protein which can be destroyed by heat or proteolytic enzymes (6, 7). It is active in animals that are tolerant to endotoxin and also has a shorter latency period than endotoxin (4).

To better understand why the rat responds to endotoxin injections with a decrease in body temperature, we have investigated the following: (a) the response to low doses of endotoxin; (b) the effects of repeated daily injections; (c) a possible leukocytic pyrogen in the rat; (d) the response of the rat to leukocytic pyrogen from species in which endotoxin will produce a fever.

Materials and Methods. Female Holtzman rats (180–220 g) were fed Purina lab chow and water *ad libitum*. They were maintained in animal quarters at 70 ± 2°F with 12 hr of light (6 a.m.– 6 p.m.) and 12 hr of darkness (6 p.m.– 6 a.m.). Endotoxin from *Escherichia coli* 055:B5, lot no. 471330, was purchased from Difco Laboratories, Detroit, Michigan.

Leukocytic pyrogen was prepared by a slight modification of the method of Kaiser and Wood (8). Precautions similar to those they employed were taken to avoid bacterial contamination or extraneous endotoxin. If an exudate was contaminated with bacteria, the results were discarded. Exudates were obtained after intraperitoneal infusion (50 ml in rats or 500 ml in rabbits) of saline containing 100 mg percent shellfish glycogen (Mann Research Laboratories, Inc., New York, N. Y.), 0.5 g/liter of streptomycin, and 100,000 units/liter of crystalline penicillin G. The exudates were collected with a heparinized syringe 16 hr after infusion and filtered through gauze into an iced flask containing heparin (20 units/100 ml of exudate). The leukocytes in the filtrate were counted, spun down in the cold at 800*g* for 10 min, and then resuspended in saline. Incubation was started immediately and continued for 5 hr with gentle shaking at 37°. The supernatant (leukocytic pyrogen) was separated by centrifugation; that not used immediately was stored at 4°. The rabbit leukocytic pyrogen was tested for activity in rabbits which were made refractory to endotoxin (9).

Glassware and other materials used in the preparation or injection of test material were sterilized by dry heat at 170° for 3 hr to inactivate pyrogens.

There is a daily variation in body temperature of the rat (10). Handling (10, 11), depth of insertion of the temperature probe (11), and environmental temperature (12) can have a pronounced effect on the temperature obtained. We used the following precautions to minimize these variations. Body temperatures of the rats, which were immobilized in plastic restraining cages, were measured with thermistors (No. 402, Yellow Springs Instrument Co., Yellow Springs, Ohio) carefully inserted into the rectum to a constant depth of 6 cm. The room temperature was maintained at 70°F, and the tests were started at the same time each day.

Results. The body temperature of restrained rats during light or dark cycles is shown in Fig. 1. The animals in the light cycle displayed a slow, steady decline in body temperature throughout the period of restraint. Rats restrained during the dark cycle started at a higher body temperature, and the temperature decreased rapidly during the first hour. Animals restrained during day-

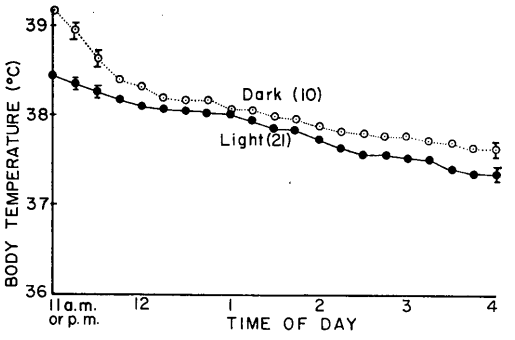

FIG. 1. Effect of restraint upon the body temperature of rats in either the light or dark cycle. The animals were placed in the restraining cages at either 11 a.m. (light cycle) or 11 p.m. (dark cycle). The brackets indicate the standard error for the number of animals indicated in parentheses.

light were, therefore, used in all of the experiments to follow.

The effects of varying doses of endotoxin on the body temperature of rats are shown in Fig. 2. A biphasic temperature curve was found with low points at approximately 1 hr and 45 min and at 2 hr and 45 min after injection. The effects of repeated daily injections of 100 μg of endotoxin are shown in Fig. 3. The first injection apparently disrupts the normal temperature cycle of the rats. When the second injection was given 24 hr later, it caused an initial increase instead of the usual decrease in body temperature. After the fourth daily injection a tolerance was developed, and the body temperature curve closely resembled the decline observed in control animals.

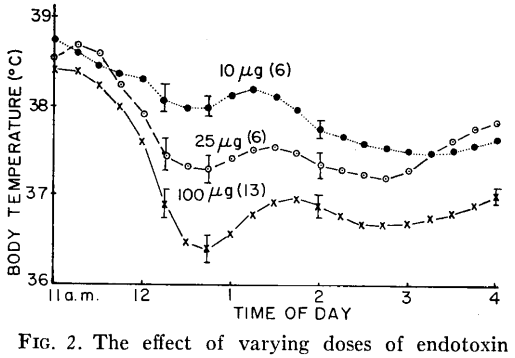

FIG. 2. The effect of varying doses of endotoxin on the body temperature of restrained rats. The endotoxin was injected ip at 11 a.m. when the rats were placed in the restraining cages.

Extracts were prepared from polymorphonuclear leukocytes from the peritoneal cavity of rabbits or rats; the effect that an extract from 2×10^8 cells had upon body temperature of the rat is shown in Fig. 4. The control protein, prepared from rabbit leukocytes which were ruptured and not incubated, and the rat leukocytic extract had no significant effect on body temperature. An extract prepared from 1×10^9 rat leukocytes also failed to alter the body temperature of rats.

Leukocytic pyrogen prepared from rabbit cells produced a rapid depression and then an increase in the body temperature of the rat. Leukocytic pyrogen caused a faster decline in

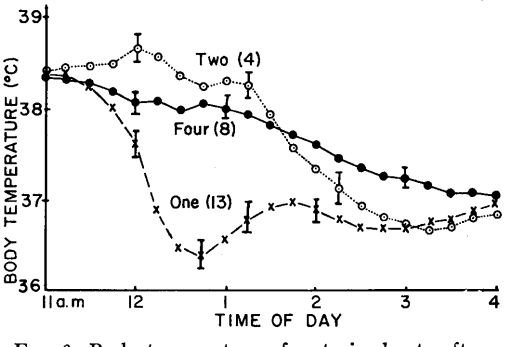

FIG. 3. Body temperature of restrained rats after a single or 2 and 4 daily injections of 100 μg of endotoxin. The brackets show the standard error for the number of animals in parentheses.

body temperature than a dose of endotoxin which produced the same minimum temperature. Other changes in the conditions of preparing an extract from rat leukocytes that did not affect the temperature when tested in rats were: (a) using starch instead of glycogen to produce the exudate; (b) removing the exudate at 5 instead of 16 hr; (c) incubation of the leukocytes in Ringer–phosphate buffer; and (d) adding small amounts of endotoxin in an attempt to activate the leukocytes.

Rabbit leukocytic extract heated at 90° for 30 min had no effect on the body temperature of rats (Fig. 5). After 6 daily injections of rabbit leukocytic pyrogen, the initial rapid depression of body temperature was still produced; but the sustained increase (Fig. 4) from 2–5 hr after injection was lost. When rabbit leukocytic extract was given to rats

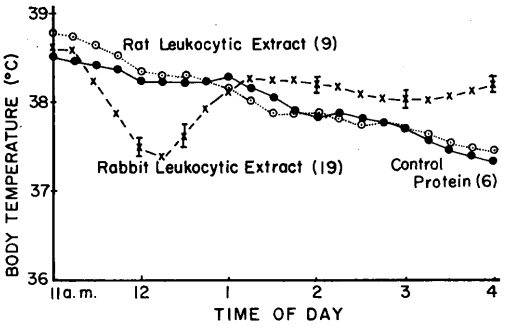

FIG. 4. Body temperature of rats after injecting leukocytic extract prepared from 2 × 10^8 rat or rabbit polymorphonuclear leukocytes. The control protein was prepared by rupturing rabbit leukocytes that were not incubated and injecting the amount of protein that was used for the rabbit leukocytic extract.

which were endotoxin tolerant (Fig. 6), the initial depression at 1 hr was retained and the increase at 2–5 hr after injection was greater than that produced in normal rats (Fig. 4).

Discussion. The data presented confirm and extend to low dosages the hypothermia produced in the rat after injections of endotoxin (1–3). Filkins and DiLuzio (3) found that when a tolerance was induced by 6 daily injections of a fairly high dose of endotoxin, the rat was tolerant to the production of hypothermia by a somewhat lower dose. We found that a tolerance could be induced rapidly by giving the same daily dose. A similar finding of a rapid and almost complete tolerance has been shown for the production of hypoferremia in the rat by endotoxin (13). Tolerance formation may be somewhat different in the rat than in the rabbit or dog (4) as indicated by the unusual temperature curve (Fig. 3) after the second daily injection of endotoxin in the rat.

Filkins and DiLuzio (3) suggest that the hypothermia produced in the rat after endotoxin may be related to the increased phagocytosis noted shortly after endotoxin injection. However, enhanced clearance after injecting endotoxin in the rat was not observed with all types of colloidal material (14), and there is some evidence that endotoxin cleared slowly in the rat (15). A more satisfactory explanation might be found, therefore, by looking for an intermediate or endogenous pyrogen. Falk (16) demonstrated a mouse leukocytic pyrogen which altered the circadi-

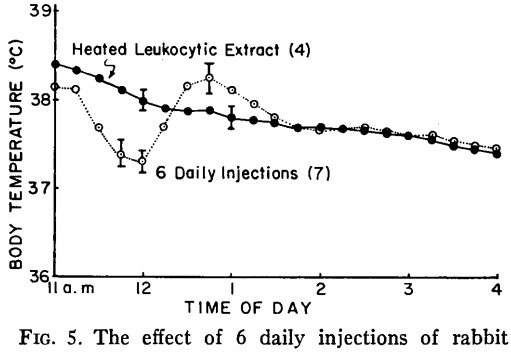

FIG. 5. The effect of 6 daily injections of rabbit leukocytic pyrogen on the body temperature of rats. The leukocytic pyrogen was inactivated by heating at 90° for 30 min.

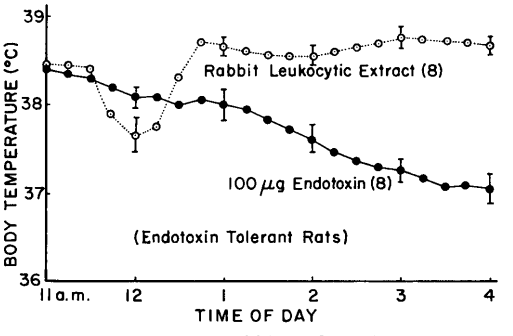

FIG. 6. The effect of rabbit leukocytic pyrogen on the body temperature of rats tolerant to 100 μg of endotoxin. The brackets show the standard error for the number of animals indicated in parentheses.

an temperature cycle of mice. In the present studies we were unable to show a leukocytic pyrogen from rat polymorphonuclear leukocytes. The extract from rat leukocytes was also ineffective in the rabbit (21). It is possible that rat leukocytes require different conditions for activation. The rat will respond to leukocytic pyrogen from the rabbit, but with hypothermia instead of a fever which is characteristic for the rabbit. Leukocytic pyrogen was effective only in the species from which it was derived (16, 17), whereas endogenous pyrogen produced fever in heterologous species (17–20). Although the rabbit leukocytic pyrogen does not produce a fever in the rat, it does show many of the other

characteristics such as, fast action, destruction by heat, and failure to develop tolerance.

There is some evidence that the rat leukocytes produced in the peritoneal cavity have been activated, since they released a factor which produced hypoferremia in tolerant rats (21). It appears that the response of the thermoregulatory center of the rat to either endotoxin or endogenous pyrogen was different than most other species.

Summary. Endotoxin even in small doses produced a biphasic hypothermia in restrained rats. A rapid tolerance was produced by daily injections of the same dose of endotoxin. Rat leukocytic extracts failed to alter the body temperature of the rat. Rabbit leukocytic pyrogen produced a rapid decrease and then an increase in the body temperature of the rat. This leukocytic pyrogen was fast acting, was effective in endotoxin tolerant rats, would not produce tolerance upon repeated injection, and was inactivated by heating at 90°.

The effective technical assistance of Mrs. Faye Jones is gratefully acknowledged.

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Received Feb. 24, 1969. P.S.E.B.M., 1969. Vol. 131.