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Fever and Release of Endogenous Pyrogen in Leukopenic Rabbits.* (29484)

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Following administration of bacterial endotoxins, rabbits develop profound leukopenia followed by fever and leukocytosis. During the period of leukopenia, the granulocytes are presumably injured by the toxin and release a humoral substance, endogenous pyrogen (EP), which acts directly on the thermoregulatory centers to produce fever(1). Support for the idea that endogenous pyrogen is derived primarily from leukocytes is based on experiments demonstrating that leukocytes themselves are pyrogenic(2), and that endogenous pyrogen is absent from the serum of animals made leukopenic with nitrogen mustard(3). These observations have been cornerstones in the hypothesis that endotoxin fever is mediated solely through the release of endogenous pyrogen. Several observations, however, suggest that endotoxins may produce fever in the absence of EP. First, leukopenic animals with no demonstrable EP may have high fever following administration of endotoxin(3). Second, animals made tolerant to the pyrogenic action of endotoxin continue to have some fever in the absence of demonstrable EP(4). Finally, tolerance to the pyrogenic effects of endotoxin is not paralleled by diminution in the leukopenia(5). The apparent dissociation between EP and fever may be in part related to the fact that the passive transfer technique measures only relatively large quantities of EP, while much less EP may be needed to activate the thermoregulatory centers in the donor. One facet of this problem which has received insufficient attention, however, is the reactivity of the leukopenic animal to endotoxin *per se,* irrespective of release of EP. For example, leukopenic animals are more sensitive to the lethal effects of endotoxin and, at times, may also have a hyperthermic response to the pyrogenic stimulus(6). The present studies reexamine the effects of different doses of endotoxin on fever, release of EP, and lethality in normal and leukopenic animals.

Materials and methods. Male and female albino rabbits weighing 2-3 kg were housed in individual cages in an air-conditioned room. Food and water were withheld on the day of experiment. Temperatures were measured with an indwelling rectal resistance coil and a Telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) every 30 minutes for 8 hours after injection of endotoxin and 3-4 hours after injection of endogenous pyrogen. White blood counts and differential counts were performed in the usual manner. The endotoxin was *S. enteritidis* (Difco Laboratories, Detroit, Mich.) suspended in pyrogen-free sterile physiologic saline in concentration of 2.0 μg/ml. Injections were made through the marginal ear vein. Nitrogen mustard (Mustargen Hydrochloride, Merck Sharp & Dohme, Philadelphia), freshly mixed in pyrogen-free saline in a concentration of 1.0 mg/ml just prior to

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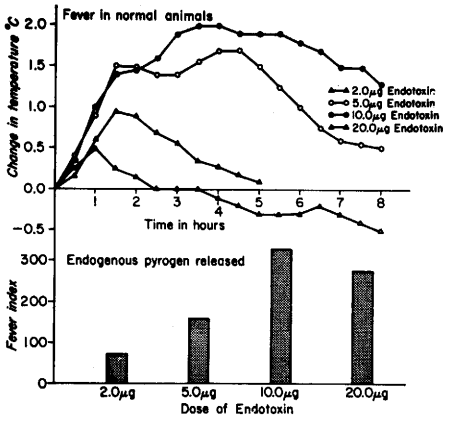

FIG. 1

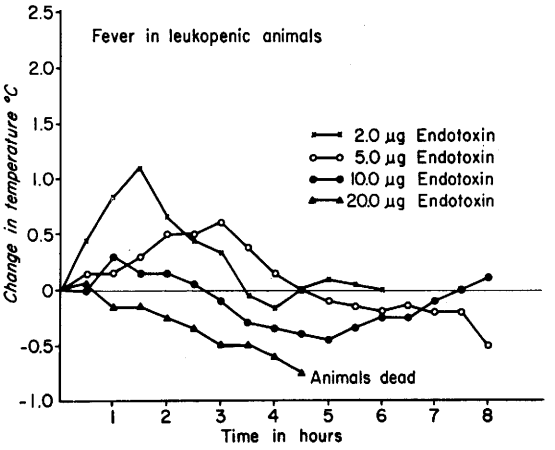

FIG. 2

FIG. 1. Fever and release of EP in normal rabbits given *S. enteritidis* endotoxin.
FIG. 2. Fever in leukopenic rabbits given *S. enteritidis* endotoxin.

injection, was administered intravenously. The initial dose of nitrogen mustard was 2.5 mg/kg. A second injection of 0.5 mg/kg was given 48 hours later to sustain leukopenia. Endotoxin was given 4 days after the initial injection of nitrogen mustard at the time of maximal leukopenia. Of a total of 88 animals injected with nitrogen mustard, 20 had *no* detectable granulocytes in the circulating blood except an occasional basophil. These animals were labelled "totally" granulocytopenic.

Endogenous pyrogen was obtained from rabbits by cardiac puncture performed 120 minutes after injection of graded doses of endotoxin. The blood was stored overnight at 4°C and tested in recipients within 2 weeks, after sterility was demonstrated by culturing the serum in thioglycollate broth. Assay of endogenous pyrogen was performed by injecting 8 ml of serum into each of 3 normal recipient rabbits. Animals used for pyrogen assay were not used if their temperatures fluctuated more than 0.2°C during a 2-hour period before the study. Fever curves were plotted on standard graph paper, and the area beneath the curve for the 2 hours after injection was measured by planimetry (Keuffel-Esser 423 M-9788). The resulting area was defined as the fever index.

All needles, glassware and instruments were sterilized at 170°C for 3 hours. All serum and other injectable agents were cultured in thioglycollate broth for 72 hours and discarded unless sterile.

Results. Relationship of endotoxin dosage to fever, lethality and production of endogenous pyrogen in normal animals. Four groups of 6 rabbits were injected with *S. enteritidis* endotoxin. Each group received one of the following doses of endotoxin: 2.0 μg, 5.0 μg, 10.0 μg or 20.0 μg. Three animals in each group were used for measurements of temperature and the remaining 3 were bled for endogenous pyrogen. Fig. 1 shows the fever curve and amount of EP released in each of the 4 groups; the lethal effects of the endotoxin are summarized in Table I. There was a direct correlation between dose of endotoxin, magnitude and duration of fever, and quantity of EP released up to a dosage of 10 μg. At the highest level of 20 μg the animals became hypothermic despite continued release of EP. At that dose of endotoxin, the

TABLE I. Death in 36 Hours After Endotoxin.

Dose, μg	Control	%	Totally leukopenic	%
2.0	0/3	0	1/3	33
5.0	0/3	0	2/3	66
10.0	0/3	0	3/3	100
20.0	3/3	100	3/3	100

animals invariably died within 24 hours following injection, while at lower dosage, endotoxin did not exert a lethal effect.

Relationship of dosage of endotoxin to fever, lethality and production of endogenous pyrogen in leukopenic animals. Four groups of 5 "totally" leukopenic rabbits were injected with *S. enteritidis* endotoxin in dosage of 2.0 μg, 5.0 μg, 10.0 μg, or 20.0 μg. Three animals in each group were used for recording of temperature and EP was assayed in the remainder. The composite fever curves obtained with injection of the different doses of endotoxin are shown in Fig. 2. At the 3 highest dose levels of endotoxin, the animals not only failed to respond with fever but appeared severely ill, with profuse diarrhea and hypothermia. Seven of the 9 rabbits were dead within 24 hours after endotoxin was given and an additional rabbit died shortly thereafter (Table I). No EP was demonstrable in any of these leukopenic animals. However, in the group of leukopenic rabbits given the smallest dose of endotoxin, 2.0 μg, the febrile response was comparable to that of normal controls. This did not depend upon the release of EP since this substance was present in normal animals who had no more fever than the leukopenics, who had no EP. Furthermore, the group of rabbits receiving the smallest dose of endotoxin was not as severely ill as those given higher doses and only 1 of 3 died (Table I). These observations indicate that leukopenic animals were more sensitive to endotoxin than normals, and that 5.0 μg of toxin exerted the same effect in leukopenic animals as 20 μg did in normals.

Discussion. It is apparent that the magnitude and duration of fever following administration of endotoxin to normal animals is largely a function of dose. While there is a progressive increment in fever with increasing dosage in normals, very large amounts of toxin are associated with hypothermia rather than hyperthermia(7). Under these circumstances the vasomotor effects of the toxin predominate, there is pooling of blood in the splanchnic circulation and the cerebral blood flow is diminished. This, in turn, sharply reduces the quantity of endogenous pyrogen reaching the thermoregulatory centers, and fever does not result. In this regard, it is important to point out that EP, which is presumably a reflection of tissue injury, was not appreciably diminished in these afebrile animals. This is similar to the situation observed following administration of aspirin which suppresses fever but does not affect release of EP(3). Presumably this drug acts by depressing the responsiveness of the thermoregulatory centers directly.

Leukopenic animals are much more sensitive to endotoxin than normals and of the toxic manifestations, hypothermia occurs at a much lower dose. Occasionally, leukopenic animals hyperreact to endotoxin with more fever than normals(3,6,8); this is probably a reflection of the type of endotoxin and dose employed. This variable reactivity of the leukopenic animal may explain some of the conflicting data obtained by others. For example, Freedman showed that leukopenic animals had more intense pyrexia than normals and that the exaggerated febrile response was proportional to the severity of the leukopenia (6). He postulated that endotoxin produced fever directly rather than by releasing EP and suggested that the absence of leukocytes failed to protect the animals from the toxic effects of endotoxin, permitting them to develop more fever. Herion *et al,* on the other hand, showed that totally leukopenic animals failed to respond to endotoxin with fever although they were more susceptible to the lethal action of toxin and postulated that the leukocyte is a necessary intermediate in endotoxin fever(9). It is not unlikely that these authors were working within dose ranges of toxin which produce hypothermia.

Our studies confirm previous work that leukopenic animals have no demonstrable endogenous pyrogen, and that these animals have no fever within a wide dose range. This is further evidence that leukocytes are the major, although probably not the only, source of EP(10). These observations also emphasize, however, that some animals with large quantities of EP may have no fever, presumably because the sensitivity of the

thermoregulatory center is depressed or because not enough circulating pyrogen reaches the center to activate it. Conversely, some animals may develop fever in the absence of EP, as was the case with leukopenic animals given relatively small amounts of endotoxin. The dissociation between EP and fever has been attributed by Gillman, Bornstein and Wood to an insufficient quantity of EP in the circulation to demonstrate by passive transfer(11). With this technique the pyrogen would be diluted in a large volume of blood and no fever would be evident. These authors argue that enough pyrogen is present in the serum of the donor, however, to activate the thermoregulatory center. It is unlikely, however, that this explanation can be invoked in the present experiments because both normal and leukopenic animals had the same amount of fever when given the same relatively low dose of endotoxin. In one instant EP was present, in the other it was not. It is clear that the magnitude of the febrile response was within the sensitive part of the dose-response curve to endotoxin (12) and was not in the hyperthermic range in which alterations in fever would be difficult to demonstrate. It is more likely that under certain restricted circumstances toxic substances such as nitrogen mustard or even endotoxin *per se* alter the blood-brain barrier (13), permitting leakage of endotoxin into the CNS. Once in the spinal fluid, the toxin may act directly on the thermoregulatory centers to produce fever(14). These studies are not presented as an argument against the hypothesis that endotoxin or other types of fever are mediated by endogenous pyrogen. However, they emphasize again that fever may occur in the absence of this substance.

Summary. In normal rabbits given endotoxin, the degree of pyrexia and release of EP are proportional to the dose of endotoxin injected, until very high dosages are reached, at which point hypothermia occurs and the animals die. In leukopenic rabbits the hypothermic and lethal effects are evident at much lower dosage. These animals produce no endogenous pyrogen. At the lowest doses tested leukopenic animals had as much fever as normals but did not release EP.

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Determination of Avidin by Use of Radioactive Biotin and Sephadex Chromatography.* (29485)

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Microbiological methods(1) have long been used for determination of avidin, the

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