Comparative Pyrogenic Reactivity of Rabbit and Man to Bacterial Endotoxin* (34059)

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Bacterial endotoxins have been administered intravenously to man under a variety of conditions: (a) during inadvertent contamination of blood or of parenteral medications; (b) for therapeutic purposes; *i.e.*, treatment of various dermatologic (1), opthalmologic (2), allergic (3), circulatory (4), neoplastic (5), and neurologic (6) disorders; (c) for assessment of bone marrow reserve (7), of the presence of chronic pyelonephritis (8), and of hypothalamic–pituitary–adrenal axis function (9); and (d) for definition of mechanisms of acquired resistance to endotoxemia (10). Since the rabbit is generally employed for preliminary testing of most biologicals and for standardizing endotoxin preparations before parenteral use in man, assessment of the relative sensitivity of man and the rabbit to the pyrogenic properties of endotoxin is of obvious importance. Studies in this laboratory over the past decade on the role of endotoxins during gram-negative bacterial infections have permitted the opportunity to compare quantitatively the reactivity of rabbits and of healthy man to the pyrogenic activity of three different preparations of purified bacterial endotoxins. Such quantitative comparative data employing healthy man have not to our knowledge been previously reported.

Methods and Materials. Volunteers for these studies were 23- to 52-year-old healthy male inmates of the Maryland House of Correction, Jessup, Maryland. Complete medical evaluations were performed to verify the fitness of each participant. Each volunteer was apprised of all aspects of the pyrogen studies. For comparisons with man, healthy New Zealand albino rabbits weighing 1.8–2.2 kg, never previously injected with endotoxin, were obtained from a uniform source.

Endotoxin preparations. To avoid extraneous pyrogen contamination, all syringes, needles, and glassware were either of the pyrogen-free disposable type or were preheated overnight at 200°C. The following endotoxins were employed: (1) *Salmonella typhosa* endotoxin, Boivin-extracted and further purified by ethanol precipitation (11)[2]; (2) *Escherichia coli* endotoxin, Boivin-extracted (Difco); and (3) *Pseudomonas* endotoxin, prepared by tryptic digestion (12)[3] Each endotoxin preparation was diluted in sterile, pyrogen-free saline and stored at 4°. All preparations were bacteriologically sterile before administration to man. Pyrogen assays were performed in rabbits 1–2 days before testing in man.

Pyrogen assay in rabbits. Animals were loosely restrained by chain collars in fiberglass stalls after acclimatization for 18–24 hr in an air-conditioned room. Room temperature was maintained at approximately 70°F, comparable to that employed for studies in man. Thermistor probes were inserted 6 in. into the rectum and connected to a recording telethermometer (Yellow Springs Instrument Co.). Temperatures were monitored 1–2 hr before pyrogen injection; animals exhibiting temperatures greater than 104°F, or varying more than 0.3°F in any 30-min control period were excluded. After endotoxin injection,

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temperature increments were monitored every 30 min until fever subsided. Three or more acclimatized rabbits were used for each assay; temperature increments were averaged and plotted as a function of time on standard graph paper.

Pyrogen assay in man. Assays were begun between 8 and 9 A.M. with the subjects confined to bed and covered with a light blanket. Breakfast was withheld and no food ingested during the study. Flexible thermistors were inserted 6 in. into the rectum. Temperatures were monitored immediately before and every 30 min for 7 hr after intravenous endotoxin injection. Three or more subjects were employed for each assay; temperature increments were averaged and plotted as a function of time in the same manner as the rabbit assay. Subjective toxic reactions were graded as follows: 0 = asymptomatic; 1+ = mild headache, anorexia; 2+ = moderately severe headache, anorexia, chilliness; 3+ = severe headache, anorexia, shaking chills, myalgia.

Results. Before pyrogenic reactivity of rabbit and man can be compared quantitatively, three major qualitative differences in species response to endotoxin, as observed during extensive studies in this laboratory, must be defined:

Diurnal temperature fluctuations. If no injections are given, no diurnal changes in mean base line temperature occur in acclimatized rabbits. In contrast, diurnal temperature fluctuations in untreated healthy men lead to gradual rises in rectal temperature, at times exceeding 1°F by mid-afternoon despite maintenance of the basal state. Mean temperature increments in groups of 3 or more fasting reclining subjects, however, are consistently under 0.5°F by 3 hr from the 8–9 A.M. starting point.

Emotional status. If pyrogen-free saline is administered intravenously or sham injections performed, emotional lability of rabbits, despite acclimatization, often evokes some rise in rectal temperature; mean temperature increments in groups of three or more animals, however, generally do not exceed 0.5°F. Such acute temperature increments secondary to emotional lability after intravenous injections are not seen in man.

Configuration of endotoxin-induced fever curves. When endotoxins are administered intravenously, febrile responses in rabbits are always of more rapid onset than in man. Small pyrogenic doses of endotoxin induce febrile responses in rabbits that peak at approximately 1.5 hr; larger doses evoke the well-known second peak, attained by approximately 3 hr. In contrast, even with large pyrogenic doses of endotoxin, little or no temperature increments are seen in healthy men before 1 hr, and peak fevers are attained only by Hour 3. From the above considerations, the following criteria are used to define threshold pyrogenic responses to endotoxin: rabbit—mean temperature increments between 0.5 and 1.0°F by 1.5 hr; man—mean temperature increments between 0.5 and 1.0°F by 3 hr from the 8 to 9 A.M. starting point.

The comparative reactivity of mature rabbits and of healthy volunteers to the pyrogenic activity of *S. typhosa, E. coli,* and *Pseudomonas* endotoxins are summarized in Figs. 1 and 2. Figure 1 compares mean reactivity in terms of micrograms per kilogram. The data indicate that for induction of threshold pyrogenic reactions, a healthy man requires approximately the same per kilogram quantity of bacterial endotoxin as does the rabbit. Thus, threshold pyrogenic doses for both rabbits and man fall between 0.00010–0.00014 and 0.0010–0.0014 μg/kg for *S. typhosa* endotoxin, and approximate 0.001 μg/kg for *E. coli* endotoxin, and 0.05–0.07 μg/kg for *Pseudomonas* endotoxin. While threshold pyrogenic doses of endotoxin are similar, man responds more vigorously than the rabbit as endotoxin dosage is increased; *i.e.,* dose-response relationships for man are appreciably steeper than for the rabbit with each endotoxin preparation tested. Subjective toxic responses in man also increased sharply as endotoxin dosage increased, paralleling the pyrogenic response.

Figure 2 indicates that when compared on a total dose basis, less endotoxin was required to elicit threshold febrile responses in the rabbit than in man. As total endotoxin dose administered was increased, however,

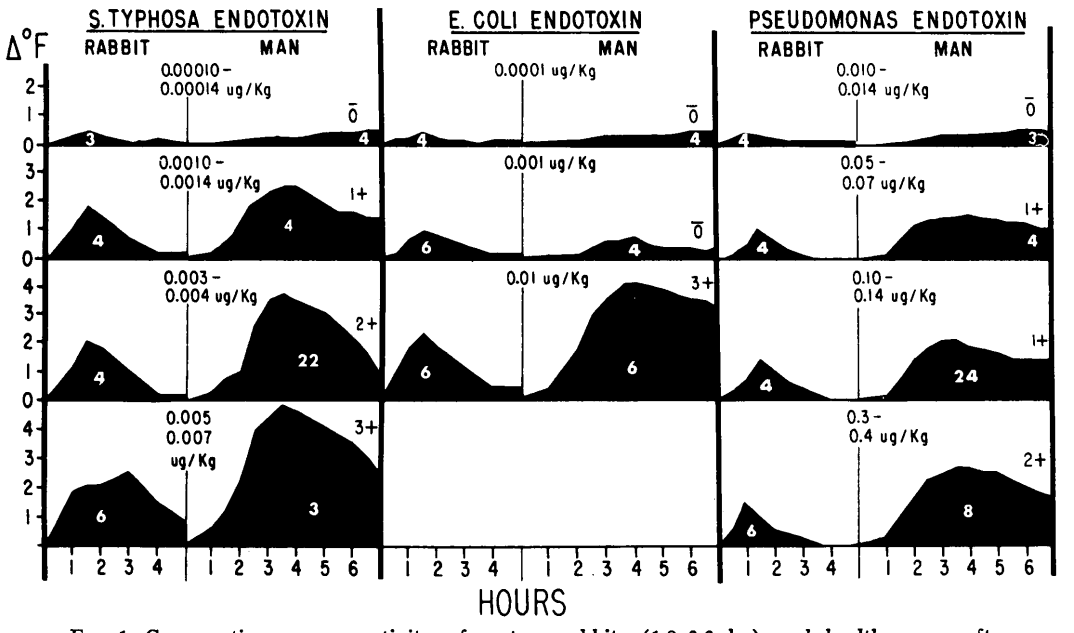

FIG. 1. Comparative mean reactivity of mature rabbits (1.8–2.2 kg) and healthy man after intravenous administration of *S. typhosa, E. coli,* and *Pseudomonas* endotoxins on a micrograms per kilogram basis. Number of subjects in each study are indicated within black febrile area. Subjective toxic reactions of man are graded 0 to 3+. (See text for explanation of grading).

febrile responses of man rapidly approached and then exceeded those of the rabbit.

Discussion. Although the rabbit is used extensively for detecting endotoxin contamination of biologicals employed in man, scant data are available on comparative endotoxin reactivity of rabbit and man. In 1925, Seibert (13) observed that intravenous administration of 1.0–1.5 ml/kg crude bacterial filtrates induced low-grade febrile responses both in mature rabbits and in several patients with a variety of illnesses. Of interest, one patient with hepatic cirrhosis responded with appreciably more fever than the rabbit; that such hyperreactivity is characteristic of patients with cirrhosis, however, was not recognized until 24 years later (14). In 1942, Co Tui and Shrift (15), using larger quantities of crude bacterial pyrogens presented evidence that one patient was at least three times as sensitive to such pyrogen (4.1°C rise after 11 ml/kg) as was a rabbit (2.2°C rise after 30 ml/kg). In 1954, Dare and Mogey (16) concluded that on a microgram per kilogram basis, the normal rabbit was seven times more susceptible to threshold pyrogenic doses of bacterial pyrogen than man. These latter studies, however, employed symptomatic, *i.e.,* uncontrollable chilling, rather than febrile responses to determine threshold pyrogenic doses for man, whereas minimal fever-evoking doses were selected for the rabbit. In 1956, Westphal stated that according to his experience, the minimal pyrogenic dose (μg/kg) of purified *Salmonella abortus equi* endotoxin was practically the same for rabbits and for humans (17). In 1961, Keene, Silberman, and Landy used purified *Serratia marcesens* endotoxin and documented the febrile responses after a low dose (0.03 μg/kg) in four subjects with various cancers and a high dose (0.2 μg/kg) in two other such subjects. They reconciled the findings of Seibert and Westphal and of Co Tui and Shrift by concluding that man and rabbit have the same approximate threshold to pyrogenic stimulation by endotoxin, but that larger doses may be more pyrogenic and more toxic for man (18). The present studies, employing three different purified endotoxin preparations and healthy volunteers, substantiate these conclusions. The findings

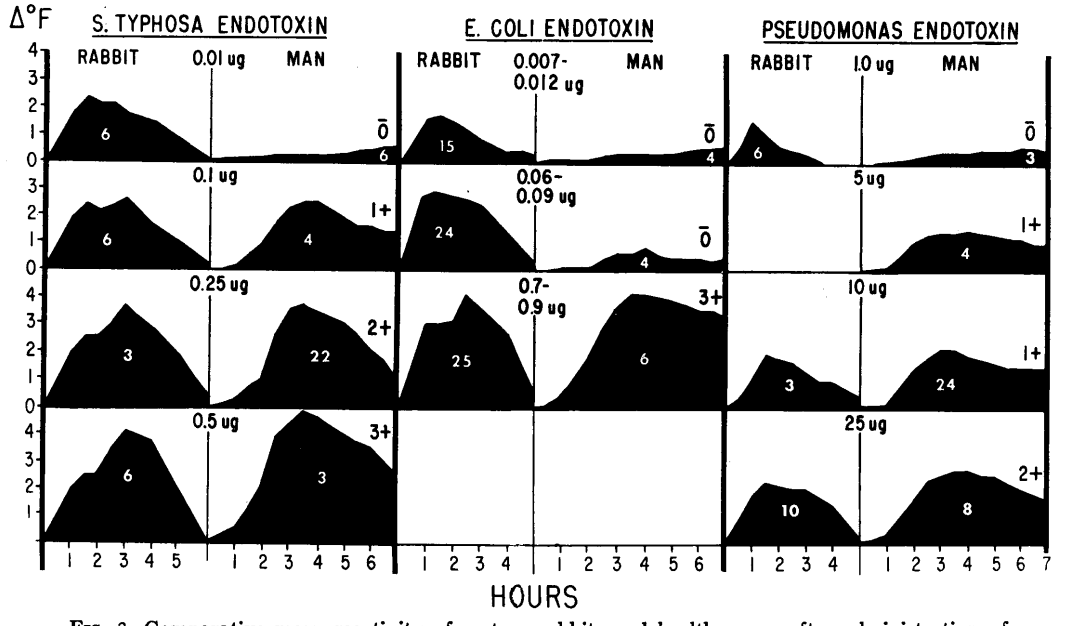

FIG. 2. Comparative mean reactivity of mature rabbits and healthy man after administration of same endotoxins depicted in Fig. 1 but expressed on a total dose basis.

indicate that for induction of minimal but unequivocal pyrogenic reactions to purified *S. typhosa, E. coli,* and *Pseudomonas* endotoxins, a healthy man requires approximately the same endotoxin dosage per kilogram body weight as does the rabbit. While threshold pyrogenic doses are virtually comparable, dose-response relationships for man are considerably steeper than those for the rabbit with each of the endotoxin preparations tested. Moreover, subjective toxic responses of man increase sharply as endotoxin dosage is increased, paralleling the pyrogenic responses. It is emphasized that if total, rather than per kilogram, endotoxin dosage is considered, the rabbit responds with unequivocal febrile reactions to lower quantities than man. The dose-response relationships being steeper in man, however, as total endotoxin dosage is increased, febrile responses of man rapidly approach and then exceed those of the rabbit. It is also emphasized that the comparative dose-response relationships herein described are applicable only to mature rabbits and healthy men. Immature rabbits are significantly more resistant to the pyrogenic activity of endotoxin than mature animals (19). Moreover, during certain human illnesses: *i.e.,* overt phase of typhoid fever and tularemia (20), brucellosis (21), and hepatic cirrhosis (14) enhanced pyrogenic and subjective toxic responsiveness develops to endotoxins; during other illnesses: *i.e.,* chronic pyelonephritis (22), malaria (14), and the convalescent phase of typhoid and paratyphoid fevers and of tularemia (20, 23) decreased responsiveness occurs. With these reservations, the present data provide a more definitive base line for interpreting those studies with bacterial endotoxins that involve extrapolation of rabbit febrile responses to man.

Summary. Comparative reactivity of mature albino rabbits (1.8–2.2 kg) and of healthy man to the pyrogenic activity of purified *S. typhosa, E. coli,* and *Pseudomonas* endotoxins is presented. On a per kilogram basis, rabbit and man are approximately equally reactive to threshold pyrogenic quantities of endotoxin. When larger doses of endotoxin are employed, the dose-response relationships become considerably steeper for man. On a total dose basis, rabbits require smaller quantities of endotoxin to elicit threshold febrile responses, but as total toxin dose is increased, febrile responses of man

rapidly exceed those of the rabbit. Subjective toxic responses of man parallel the pyrogenic responses. These data provide a more definitive base line for interpreting studies with bacterial endotoxin that involve extrapolation of rabbit febrile responses to man.

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