# SPECIES SPECIFICITY OF LEUKOCYTIC PYROGENS*

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Under appropriate stimulation, rabbit polymorphonuclear leukocytes can form and release a small neutral protein which appears to act on hypothalamic thermoregulatory centers to raise body temperature. This protein, leukocytic pyrogen, is clearly distinguished from the common contaminating lipopolysaccharide pyrogen, bacterial endotoxin, by its chemical, physiological, and pyrogenic characteristics (1–5). In the rabbit, a single intravenous injection of leukocytic pyrogen produces a distinctive fever curve which reaches a peak 35–45 min after injection and falls smoothly to the preinjection baseline within 3 hr (6). Endotoxin, in any dose and however incubated, in the absence of leukocytes, cannot reproduce this response, as fevers produced by endotoxin do not peak before 55–80 min (5, 7). Pyrogenic responses similar to those due to leukocytic pyrogen have been noted, however, after the transfer of serum obtained during the height of experimental fevers induced by bacterial infection (8), by endotoxin administration (9, 10), by immediate (11) or delayed hypersensitivity reactions (12, 13), by viremia (14–16), by fungemia (17), or by the administration of certain macromolecules (18). These circulating serum pyrogens of host origin, or endogenous pyrogens, once considered solely of granulocytic origin, may in some cases represent the product of mononuclear cell stimulation, since the rabbit mononuclear cell has been shown to produce a pyrogen with properties similar to that of the granulocyte (19, 20). Conceivably, some endogenous pyrogens may even be produced in other tissues (21).

Leukocytic pyrogen is neither formed nor released until the circulating leukocyte is "activated" (22–24). Granulocytes obtained from acute exudates, such as saline or glycogen-induced sterile peritonitis, appear fully activated, possibly as a result of the process of diapedesis or of substances present in the inflammatory exudate. In contrast to granulocytes obtained from peripheral blood, exudate cells will yield pyrogen in large amounts if simply incubated in normal saline at 37°C for several hr. Leukocytes obtained from peripheral blood require in vitro activation for the formation and release of pyrogen. Appropriate stimuli include low concentrations of endotoxin (22, 24), phagocytosis (23, 24), and, no doubt, other chemical, physiologic, and immunologic reactions, most of which introduce extraneous macromolecules or serum.

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The pyrogen does not exist preformed even in activated leukocytes, so that injection of whole cells, sonicates, lysates, or cell fractions of leukocytes of blood or exudate origin without prior incubation results in little or no fever (25, 27). Incubation of cells at 4°C, incubation of disrupted cells, or incubation in the presence of physiologic concentrations of potassium ion will prevent the formation and release of the pyrogen from activated exudate granulocytes, as will incubation in the presence of sulfhydryl inhibitors such as arsenite, *N*-ethylmaleimide or *p*-chlormercuribenzoate (26). Because the pyrogen is formed in the absence of exogenous amino acids and formation is not blocked by the presence of puromycin (27), it appears likely that this protein is not synthesized *de novo*, but is cleaved from a preformed inactive precursor by the action of an enzyme, possibly on esterase or protease, the action of which requires the presence of free sulfhydryl groups. This process is linked in some manner to the cell membrane and probably involves the sodium pump at this site, in view of the fact that ouabain can reverse potassium-induced blockade of the formation and release of pyrogen (28).

Leukocytic pyrogen has been studied most extensively in the rabbit. From washed-exudate granulocytes resuspended at a standard cell concentration, potent solutions of crude leukocytic pyrogen can be prepared which are relatively free of extraneous macromolecules and remarkably uniform in activity from lot to lot. With such preparations, purification of this protein has been undertaken[1] (29, 30) and quantitative aspects of the febrile response have been examined (6).

To date, only leukocytic pyrogen (LP) from the rabbit has been prepared in this manner. Sterile pleural or peritoneal exudates, or whole cells from such exudates, have been used as sources of canine LP (31) and of human LP (32). In these studies in which the activation and release of the leukocytic pyrogen would have been poor in comparison with the rabbit system, the fevers that were recorded have been complex and have not resembled the leukocytic pyrogen fever curve as known in the rabbit. Species specificity of leukocytic pyrogen has been examined by injection of such unprocessed canine exudates and exudate granulocytes into rabbits, and the injection of rabbit peritoneal exudates into dogs. No effect was demonstrated when heterologous preparations were used, and leukocytic pyrogen was considered to be completely species-specific in its activity (31).

The present studies were undertaken to determine whether leukocytic pyrogen could be isolated from species other than the rabbit by the same simple methods, to see if the fever curve so characteristic in the rabbit would differ in larger animals, and to investigate further the species specificity of leukocytic pyrogen. Since this work was begun, a type of leukocytic pyrogen has been prepared from human blood leukocytes, both polymorphonuclear and mononuclear cells, after activation in vitro by phagocytosis or by stimulation with endotoxin. Both preparations were shown to produce fever curves in the rabbit (24, 33, 34) that resembled those induced by rabbit leukocytic pyrogen.

## *Materials and Methods*

Methods for maintaining glassware and solutions free of bacterial endotoxin, for induction of glycogen-saline peritonitis, for processing the exudates and preparing crude leukocytic

[1] Bornstein, D. L. Unpublished data.

pyrogen, and for fever testing in trained rabbits have been described in detail in previous reports (6, 10).

Cats were 5–10 lb adults of mixed breeds which had received distemper and feline pneumonitis vaccines. Dogs were 15–35 lb mongrels in good health. Goats were young castrated males. These species were kept in cages or stalls unrestrained during the recording of temperatures. Injections were made into the cephalic veins of dogs and cats under local lidocaine anesthesia, and into the jugular veins of goats and sheep. Temperatures were recorded in these species, as in the rabbit, with thermistor probes (Yellow Springs Instrument Co., Yellow Springs, Ohio, series 401) at 5 min intervals for the 1st hr after injection, and at 5–15 min intervals for the next 2 hr or more. The fever index for the first 60 min ($FI_{60}$) is the area in square centimeters beneath the fever curve determined by planimetry, with 4 cm on the ordinate representing 1°C, and 3 cm on the abscissa representing 1 hr. In previous studies, it has been shown that the temperature response to leukocytic pyrogen is equally well characterized by the peak temperature elevation or the $FI_{60}$ (6). For comparable peak temperature elevations, feline fever curves yield somewhat higher $FI_{60}$ values than those of rabbits, due to slightly more rapid responses to the pyrogen in this species. Peak temperature rises are probably the preferable comparator.

Sterile peritonitis was induced by infusing solutions of 0.1% shellfish glycogen in pyrogen-free normal saline into the peritoneal cavity, and the exudate was harvested 12–15 hr later (6). Initially 400 ml of 0.1% glycogen were infused into each rabbit; recent modifications include increasing the volume of solution infused to 600 ml, raising the concentration of glycogen to 0.15%, and omitting the use of antibiotics in the infusate. Infusions in the cat were identical to those employed in the rabbit. The dog and goat required larger infusion volumes of 1200–1800 and 6000 ml, respectively.

The effusions were harvested and processed similarly in all species. The animal was sacrificed with intravenous pentobarbital and the abdomen was opened carefully with retraction, so that the peritoneal fluid was not contaminated by contacting the edges of the wound or any nonpyrogen-free instruments. The exudate was aspirated in a pyrogen-free system into flasks containing disodium EDTA (ethylenediaminetetraacetate) (final concentration 0.25% w/v) to prevent clumping of leukocytes and clotting. The exudate was filtered through baked cotton gauze, mixed well, sampled for culture and for cell counts, and was then centrifuged at 900 *g* for 15 min. The well-drained, or preferably, saline-washed cell pellet was next resuspended in pyrogen-free saline at a concentration of $70 \times 10^6$ cells per ml and incubated at 37°C with gentle shaking. In the initial studies, 18 hr incubations were employed, but more recent preparations have been incubated for only 2 hr because almost all the pyrogen is released in this time. After incubation, the saline-cell mixture was centrifuged at 900 *g* for 15 min and the supernatant fluid, which contained the crude leukocytic pyrogen, was decanted. If mixing, sampling, and cell counting are accurate, this crude product contains the extract of 70 million leukocytes per ml; ½ ml of such solution can be said to represent 35 million cell equivalents (MCE) of pyrogen, a convention we shall use in discussing amounts of pyrogen employed in various experiments. Crude leukocytic pyrogen prepared in this way is remarkably constant in potency from lot to lot and is stable for several months at 4°C.

Endogenous pyrogen (EP) was induced in rabbits, cats, and dogs by the intravenous injection of 25, 25, and 100 μg of *Salmonella typhosa* lipopolysaccharide (No. 0901, Difco Laboratories, Detroit, Mich.) respectively. The animals were bled 2 hr after injection from the heart or femoral vein, and the serum, which contains the endogenous pyrogen was separated. In some cases rabbit endogenous pyrogen was prepared by heparinizing the blood (10 U/ml) and collecting plasma rather than serum (EPP).

Because repeated injections of heterologous proteins can produce both hypersensitivity and circulating antibody, either of which is capable of inducing fever (11, 12), recipients were injected with heterologous material only 3 times or less, and with never more than 3 days

supervening between the first and last injections of material from a given heterologous species. In some early experiments in which by error this precaution was not followed, late fevers and markedly irregular defervescence were noted.

## RESULTS

*Induction of Peritonitis.*—The effectiveness of glycogen-induced peritonitis as a source of granulocytes in high yield and with minimal contamination by erythrocytes, other blood cells, and serum proteins, well documented in the rabbit, has been confirmed in the dog, cat, and goat. In these experiments, the donors were used only once, with open-operative collection of the exudate to prevent inadvertent perforation of bowel or blood vessels and consequent

TABLE I

*Results of Intraperitoneal Infusion of Glycogen in Physiologic Saline in Several Species*

| Species | No. of animals | Volume of infusate | Concentration of glycogen | Mean leucocyte recovery per animal |
|---|---|---|---|---|
| | | *ml* | % | |
| Rabbit | >1000 | 400 | 0.1 | $1.75–2.0 \times 10^9$ |
| | 70 | 600 | 0.15 | $3.3 \times 10^9$ |
| Cat | 8 | 400 | 0.1 | $1.53 \times 10^9$ |
| | 5 | 500 | 0.15 | $2.49 \times 10^9$ |
| Dog | 6 | 1200–1800 | 0.1–0.15 | $12.3 \times 10^9$ |
| Goat | 2 | 6000 | 0.15 | $20.5 \times 10^9$ |

Exudates recovered after 12–15 hr contain 90–98% granulocytes

contamination with endotoxin or whole blood. Peak cellular yields were found at 12–15 hr. In Table I, the cellular yields in these various species are tabulated.

*Release of Leukocytic Pyrogen.*—The exudate cells, washed once with saline, were resuspended at a concentration of $70 \times 10^6$ per ml in pyrogen-free saline and incubated at 37°C with mild shaking for 2–4 hr. The supernatant fluid was separated after centrifugation at 900 *g* for 15 min, and the cells were resuspended and reincubated for longer time periods, or were used for other purposes. Both the initial and the subsequent incubation supernatants were tested. In all species, the pyrogen had been released almost completely in the first incubation, as has been shown in the rabbit. In all four species studied, therefore, exudate granulocytes appear activated and can release LP upon brief incubation in normal saline at 37°C.

*Response to Homologous Pyrogen:*

*Rabbit.*—The rabbit response to homologous LP has been studied in some detail (6) and is summarized in Table II. From many observations, a dose of

$17.5 \times 10^6$ cell equivalents (MCE) has been found a convenient standard test dose level in the rabbit, with LP prepared in this manner. In over 100 observations in 30 recipients with different preparations of LP, a mean temperature elevation of 0.9°C (± 0.3°C) was noted between 35 and 50 min after intravenous injection, with prompt defervescence to baseline by 3 hr, giving rise to a 60 min fever index of about 6.5 $cm^2$ (±2.0). Marked variations in sensitivity can be seen among rabbits, but the responses of a trained recipient are quite stable. For accurate comparison of potency of two different samples or preparations

TABLE II

*Response of Cat, Rabbit, and Dog to Homologous Leukocytic Pyrogen*

| LP dose | Cat | | | Rabbit | | | Dog | | |
|---|---|---|---|---|---|---|---|---|---|
| | | dT | $FI_{60}$ | | dT | $FI_{60}$ | | dT | $FI_{60}$ |
| | | °C | | | °C | | | °C | |
| $3.5 \times 10^6$ | (10) | 0.85 | 6.7 | | | | | | |
| $17.5 \times 10^6$ | (22) | 1.15 | 9.4 | (>100) | 0.90 | 6.5 | | | |
| $10 \times 10^6$ | | | | (33) | 0.73 | 5.8 | | | |
| $20 \times 10^6$ | | | | (40) | 0.99 | 7.2 | | | |
| $40 \times 10^6$ | | | | (27) | 1.19 | 8.8 | | | |
| $100 \times 10^6$ | | | | (24) | 1.33 | 9.6 | | | |
| $70 \times 10^6$ | | | | (11) | 1.41 | 10.1 | (5) | 0.25 | 2.0 |
| $175 \times 10^6$ | | | | | | | (4) | 0.98 | 7.8 |
| $350 \times 10^6$ | | | | | | | (6) | 1.25 | 10.0 |
| $700 \times 10^6$ | | | | | | | (7) | 1.21 | 9.7 |

Number of injections at each dose level are recorded in parentheses. The rabbit data for 10, 20, 40, and 100 MCE are taken from reference 6 and represent studies in one group of 12 recipients with one lot of LP.

of leukocytic pyrogen, one should test in the same group of recipients, or if not possible, in another group matched to the first in sensitivity.

*Cat.*—Cats appear to be 2- to 4-fold more sensitive to homologous LP than the rabbit. (Table II) The responses of the cat to 3.5 MCE and 17.5 MCE of cat LP are approximately equivalent to those of the rabbit to 14 MCE and 40 MCE of rabbit LP, respectively.

*Dog.*—The dog exhibits a higher threshold dose requirement than the cat or rabbit, reflecting, but only in part, the greater size of the recipient. Only one of five dogs tested at a dose of 70 MCE developed any clear-cut temperature rise (0.7°C), but marked shivering was noted in three others, suggesting that although a pyrogenic stimulus was received, it was inadequate to overcome the heat dissipating mechanisms of these dogs. At doses of 175 MCE and above,

classic LP fever curves were obtained, except in a rare dog whose responses were markedly depressed at quite large doses (e.g., 0.55°C elevation at a dose of 1400 MCE), probably due to excitement or illness. From the data in Table II, it appears that dogs require roughly the product of 8- to 12-fold more leukocytes than does the rabbit for an equivalent febrile response.

*Goat.*—Classical LP fevers were produced in a few trials in the goat, although doses of about 1,400 MCE were required for a fever response of over 1°C. Temperature peaks occurred at a mean of 42.5 min in four experiments.

In summary, each of the four species responded to its homologous LP with a brisk monophasic fever which reached a peak at about 40 min and fell smoothly to baseline in 2–3 hr. There were species differences in the degree of sensitivity to homologous pyrogen, based on the number of cell equivalents required for

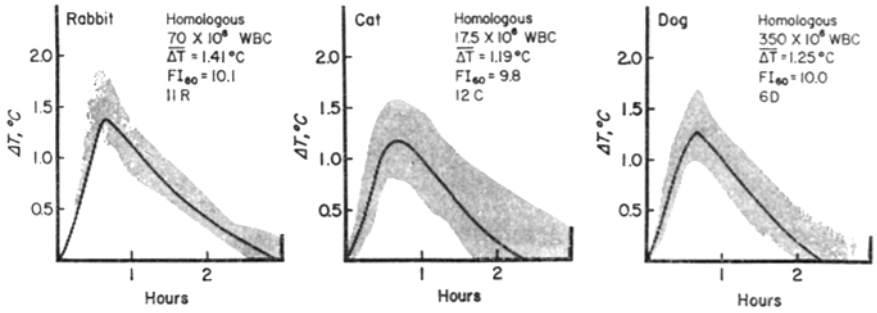


FIG. 1. The response of the rabbit, cat, and dog to homologous leukocytic pyrogen. The shaded area represents all the points for 11 rabbit, 12 cat, and 6 dog fever curves. The heavy line represents the mean of the group.

comparable effects. In addition, the cat, dog, and goat demonstrated frank shivering in the early period of temperature rise, a response not apparent in the rabbit. Typical fever responses of the rabbit, cat, and dog to homologous LP are displayed in Fig. 1.

*The Response of the Rabbit to Heterologous LP.*—Rabbits respond to both cat and dog LP with fever curves of a shape identical to those produced by rabbit LP. Although the responses are qualitatively similar, there are clear quantitative differences. Rabbits respond to either heterologous pyrogen with only about 8–12% effectiveness, relative to homologous LP preparations. In addition, as seen in Table III, increasing doses of heterologous pyrogen give appropriately increased temperature responses. Mean peak rises of 0.56°C, 0.98°C, and 1.20°C in the rabbit were noted after doses of 70, 175, and 350 MCE of dog LP. (Fig. 2). Similar dose-related responses were noted with cat LP. (Table III)

*The Response of the Cat and the Dog to Heterologous LP.*—Cats and dogs are

less satisfactory subjects than rabbits for studies of experimental fever. Rabbits can be injected with a minimum of handling, can be easily trained and handled in large numbers in convenient stalls, and will retain their probes quite well. Cats or dogs require more handling at the time of injection, are most excitable,

TABLE III

*The Response of the Rabbit to Heterologous Leukocytic Pyrogen*

| Source | Dose | Mean fever rise | Mean $FI_{60}$ | Homologous LP dose equivalent | Efficiency |
|---|---|---|---|---|---|
| | | °C | | | % |
| Dog | (13) $70 \times 10^6$ | 0.56 | 4.1 | $7 \times 10^6$ | ~10 |
| | (7) $175 \times 10^6$ | 0.98 | 7.4 | $20 \times 10^6$ | ~12 |
| | (6) $350 \times 10^6$ | 1.20 | 9.3 | $40 \times 10^6$ | ~12 |
| Cat | (14) $175 \times 10^6$ | 0.82 | 6.2 | $13 \times 10^6$ | ~8 |
| | (8) $350 \times 10^6$ | 1.05 | 8.4 | $25 \times 10^6$ | ~8 |

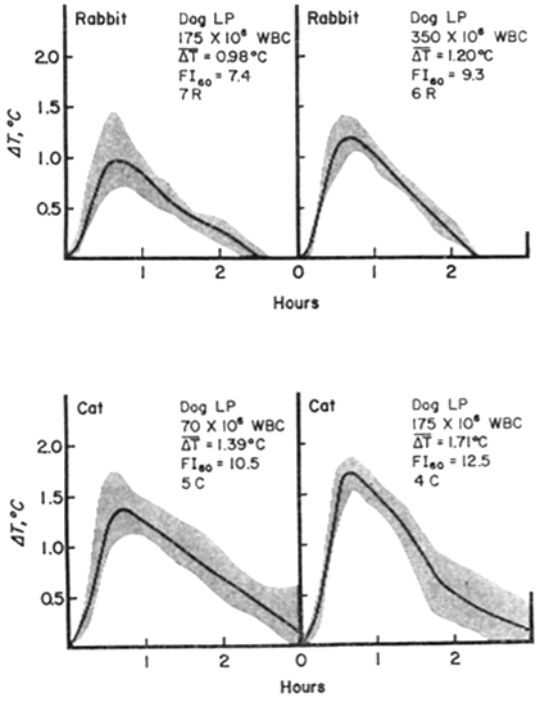


FIG. 2. Response of rabbit and cat to canine leukocytic pyrogen. The cat demonstrates greater sensitivity with this pyrogen. The responses of the rabbit and of the cat are dose related.

present problems in retaining their probes, and are less easily trained in large numbers. Intravenous injections in these species require a significant amount of handling and restraint. This interaction, and the occasional need to inject irritating or vasoactive materials, can give rise to artefactual or trivial temperature responses, unrelated to the action of leukocytic pyrogen. It is necessary that such responses be separated from significant pyrogenic responses. Because homologous leukocytic pyrogen curves were as clear-cut in these species as in the rabbit, there was no difficulty in establishing guidelines for a response to leukocytic pyrogen. All uncomplicated leukocytic pyrogen curves in the monophasic dose range (6) reach a peak between 30 and 50 min, so that fever responses which peak before 30 min or after 55 min following intravenous infection of test material cannot be accepted as a leukocytic pyrogen response, but represent instead some superimposed or different kind of physiologic disturbance. Smooth defervescence from the fever peak to baseline, appropriate response to increasing or decreasing the dose of material injected (as long as one is below the hyperthermic ceiling), and other correlative physiologic evidence of leukocytic pyrogen action such as shivering, piloerection, blanching of the skin, or other evidence of adrenergic discharge, are all strong confirmatory evidence that an accompanying temperature rise is related to injection of a pyrogen. The presence of endotoxin in the injected material is demonstrated by a late temperature peak, always 60 min or later in our experience, and either a clear second temperature peak or delayed or incomplete defervescence by 3 hr. Finally, in order to study only significant temperature variations beyond the vagaries of changing baseline temperatures, an arbitrary level of 0.7°C rise within 30–50 min, with an $FI_{60}$ of over 5.0, was set as a test for significance when cats and dogs were used as recipients.

The cat is very poorly responsive to rabbit LP. Although able to respond to as little as 3.5 MCE of homologous LP with a respectable mean fever rise of 0.85°C, the cat showed no clear response to rabbit pyrogen in doses of 175–2100 MCE in the first 41 trials. Although there were a few responses with an $FI_{60}$ of 5.0 $cm^2$ or above, only 2 of 41 curves even faintly resembled an LP fever curve and the elevations were offset by an almost equal number of nonspecific falls in temperature. The lack of responsiveness of the cat was not due to rapid destruction of the rabbit pyrogen by feline serum factors because incubation of rabbit LP in cat serum for 1 hr at 37°C did not reduce its effectiveness in the rabbit. From these data, it appeared that the cat was completely unresponsive to rabbit LP. Subsequent testing demonstrated that the cat can respond to rabbit LP, although at very low efficiency relative to homologous pyrogen. These findings are demonstrated in Table IV. The mean response in seven cats to 175 MCE of rabbit LP was 0.38°C, but at a dose of 1400 MCE all cats responded with frank shivering and a classic LP curve, with a mean peak response of 1.40°C at 35–40 min. Compared with the response to homologous material, this result represents an efficiency of about 1–2.5%.

The failure to demonstrate feline responses to massive doses of rabbit LP in the earlier studies is difficult to explain. The earlier experiments were carried out with rabbit LP which had been incubated for 18 hr, whereas in the later and successful trials we employed 2 hr–incubated pyrogen. The latter preparation has equal pyrogenicity with, but only about 20% of the protein content of the 18 hr–incubated pyrogen. It is conceivable that other granulocytic products in the 18 hr–incubated preparation disturbed the cats or interfered in some way with the development of fever in the earlier experiments. Although different recipients were used in the two experimental periods, there were no known differences in the makeup of the two groups of cats, and the handling, injection, and recording conditions and personnel were identical.

On the other hand, cats responded briskly and without ambiguity to dog LP. (Table IV) A dose of 70 MCE of canine LP produced a mean peak rise of

TABLE IV

*The Response of the Cat to Heterologous Leukocytic Pyrogen*

| Source | Dose | Mean fever rise | Mean $FI_{60}$ | Homologous LP dose equivalent | Efficiency |
|---|---|---|---|---|---|
| | | °C | | | % |
| Dog | (5) $70 \times 10^6$ | 1.39 | 10.5 | $35–70 \times 10^6$ | 50–100 |
| | (4) $175 \times 10^6$ | 1.71 | 12.5 | $87.5–175 \times 10^6$ | 50–100 |
| Rabbit | (6) $175 \times 10^6$ | 0.38 | 3.35 | — | — |
| | (2) $350 \times 10^6$ | 0.78 | 6.7 | $3.5 \times 10^6$ | ~ 1 |
| | (3) $700 \times 10^6$ | 1.03 | 7.6 | $\sim 7 \times 10^6$ | ~ 1 |
| | (5) $1400 \times 10^6$ | 1.40 | 11.1 | $\sim 35 \times 10^6$ | ~2.5 |

1.39°C in 5 cats at 43 min, with a $FI_{60}$ of 10.5 $cm^2$ (Fig. 2B) This response corresponds in effectiveness to a dose of between 35 and 70 MCE of homologous LP, indicating an efficiency of about 50–100% for canine LP in cats.

In view of the relatively large amount of homologous LP required for fever in the dog and the decreased sensitivity of the rabbit to heterologous LP, it was appreciated that very large amounts of heterologous LP might be required to determine whether dogs could respond to rabbit LP. Rabbit LP, both 18 hr and 2 hr–incubated preparations, concentrated 5- to 10-fold by lyophilization and dialyzed against normal saline, was injected into 8 recipients in doses ranging from 1400 MCE to 5100 MCE. Despite these massive doses, no temperature response of any magnitude was noted, nor was any clear-cut shivering detected which might have implied some slight LP effect. If rabbit LP were effective in the dog at as little as 2% efficiency, relative to homologous LP, we should have observed some clear effect at these doses. In light of our experience with the cat it is conceivable, even likely, that some higher dose of rabbit LP would be adequate to produce fever in the dog. Because we would like to demonstrate not only a response, but also an increase in the response

with an increase in dose of LP, preferably in enough dogs to compare this response to homologous response, and because the efficiency of rabbit LP in the dog may be even lower than in the cat, we have opted to conserve for more urgent uses the prohibitively large doses of rabbit LP that would be required.

In several trials, the dog appeared quite sensitive to cat LP, giving responses indicative of 50–100% efficiency relative to homologous LP.

*Responses of the Goat and Sheep to Heterologous LP.*—In a single trial, a dose of 1400 MCE of dog LP was found to be effective in one goat and one sheep (0.7°C and 0.6°C peaks at 40 and 35 min, respectively). In both cases, fever was associated with early shivering. Neither temperature response nor shivering was noted in the same receipients, however, after an injection of 1400 MCE of rabbit LP.

## DISCUSSION

Intraperitoneal infusion of glycogen in saline elicits heavy polymorphonuclear exudation at 12–15 hr in the rabbit, cat, dog, and goat. The washed exudate granulocytes from all four species are activated in regard to pyrogen production and will form and release leukocytic pyrogen on simple incubation with saline. The response to homologous leukocytic pyrogen is similar in all species studied, with peak response being reached between 30 and 50 min and with smooth defervescence to baseline by 3 hr. In the cat and dog, and presumably in most mammals, the febrile response varies appropriately with the dose of pyrogen administered over a sensitive range, and eventually reaches a hyperthermic palteau, such as has been demonstrated in the rabbit (6).

The development of fever is preceded by blanching and coolness of the skin, piloerection, and in cat, dog, goat, and sheep, by frank shivering. All the peripheral signs of heat conservation mechanisms were apparently stimulated in the hypothalamus by the action of the pyrogen. After temperature has risen in response to LP, heat loss mechanisms are stimulated by homeostatic mechanisms with the development of flushed warm skin, panting, and susequent defervescence.

Of the four species studied, the cat appears to be the most sensitive to homologous leukocytic pyrogen if we can assume that the amount of pyrogen released per cell in each species is comparable. This assumption can not be examined directly by measuring fever responses, since some species-specificity is operative, but might be assessable if a specific immunoassay or an enzymatic assay for leukocytic pyrogen were available. By the indirect evidence of comparing feline and canine LP in the cat, the dog, and the rabbit, it would appear that the cat and the dog release about the same amount of pyrogen per cell, since cell-standardized LP preparations are almost equally effective in each and are almost equally effective, albeit at low efficiency, in the rabbit. The amount of protein released per cell is roughly comparable in all species.

In examining the responses to heterologous LP, it is clear that the rabbit

responds with decreased efficiency to cat, dog, and goat LP, and that cat, dog, and goat respond even more poorly, if at all, to rabbit LP. On the other hand, cat and dog LP reveal a high degree of cross-compatibility. Since rabbit LP is known to be a small neutral protein of 10,000–15,000 mol wt and since LP can be prepared by the same simple technique from leukocytes of the dog, cat, and goat, it would appear that this protein is an effector molecule common to many, if not all, mammalian species. The evidence for some species specificity in activity is compatible with species-specific amino acid substitutions in a common mammalian protein, analogous to those known in insulins, growth hormones, cytochromes, and others (35). Current attempts to isolate and characterize the pure pyrogens and to develop an immunoassay may eventually allow comparison of the structure of these proteins by more direct methods such as antigenic behavior and physical and chemical studies including analyses of molecular weight, peptide maps, end group studies, and amino acid composition.

It has been reported previously that leukocytic pyrogen of the dog did not produce fever in the rabbit, but that canine endogenous pyrogens circulating in the blood during fevers caused by endotoxin or virus, could do so (31, 36). These data were used to support the hypothesis that endogenous pyrogens might be different from leukocytic pyrogen. Although this contention may or may not be proved correct when more discriminating techniques become available, there is no longer tenable evidence from species specificity data to support this contention. We have shown that canine LP prepared as described above can indeed cause fever in the rabbit. White and Petersdorf (37) have reinvestigated the species specificity of postendotoxin endogenous pyrogen. Canine and rabbit EP produced dose-related responses in the homologous species but only modest temperature responses which were not dose-dependent in the heterologous species. Normal dog or human serum was often capable of causing fever in the rabbit. It was concluded that the transfer of large amounts of foreign protein, as is required in these studies, introduces nonspecific reactions that preclude clear interpretation of the data.

We have also examined the effect of homologous and heterologous endogenous pyrogen in the dog, cat, and rabbit, using serum collected 120–150 min after an intravenous injection of endotoxin. We found interspecific studies with endogenous pyrogen much less satisfactory than comparable studies with leukocytic pyrogen for several reasons:

(*a*) For equal pyrogenic effect in the homologous host, one must transfer more than a 1000-fold excess of heterologous protein. In the rabbit, fevers comparable to those produced by 17.5 MCE of homologous leukocytic pyrogen (~100 μgm of protein) required over 2.0 ml of homologous postendotoxin serum (~140 mg of protein). This greater protein transfer increases the possibility of nonspecific reactions and leads to serum sickness, often serious or fatal, in the injected heterologous recipients, especially when larger volumes

(5–20 ml or more) are transferred, as would be required to evaluate species specificity.

(*b*) In addition to endogenous pyrogen, there may well be other vasoactive or pharmacologically active materials in the circulation in response to such a potent cellular toxin as endotoxin, which could modify or affect the febrile response.

(*c*) Quantitative study is much more difficult with endogenous pyrogen preparations which can differ considerably in potency from donor to donor, given the identical dose of endotoxin, and for which no internal standard, such as number of cells extracted or protein concentration can be used. For quantification, large pools of each type of preparation must be collected and must be extensively tested in the homologous and heterologous species.

(*d*) Finally, there appear to be some idiosyncratic responses to certain serum transfers. Normal rabbit serum given to cats frequently caused anaphylactoid responses, sometimes fatal, despite the lack of previous exposure of the cats to rabbit proteins. These responses necessitated the use of rabbit plasma for transfer of endogenous pyrogens to the cat.

In our hands it was easy to produce fevers in the rabbit with 2 hr postendotoxin serum from the cat or dog, but it was difficult, although not impossible, to produce such fevers in the cat or dog with postendotoxin plasma from the rabbit. On rare occasions, however, good responses were noted with rabbit plasma endogenous pyrogen in the cat, but some of these preparations on retesting in the same and in different cats were found inactive. In short, the data were less clear cut and less satisfactory than those involving leukocytic pyrogen preparations, but were generally consistent with them. When and if an effective method for purifying endogenous pyrogen from serum becomes available, these studies could be repeated more effectively. It is of course possible that there are some other sources of endogenous pyrogen than the granulocyte and that circulating pyrogens may represent one or more of several pyrogens, with inconstant results on this basis. Resolution of this problem will require more discriminating assay techniques.

During the course of these studies, Bodel and Atkins have reported the release from human leukocytes of a pyrogen which can give rise to typical fevers in the rabbit (24). In these studies, leukocytes were separated from circulating blood and were activated in vitro by phagocytosis of staphylococci or by stimulation with minute doses of endotoxin in a medium containing Krebs phosphate buffer, glucose, and 15% human serum. After 18 hr of incubation, but not after 5 hr of incubation, a pyrogen was detectable which would yield typical leukocytic pyrogen curves on intravenous injection into the rabbit, was equally effective in endotoxin-tolerant rabbits and in normal rabbits, and was destroyed by trypsinization. These observations are most likely due to human leukocytic pyrogen crossing species lines to work in the rabbit, presumably at low efficiency, similar to canine, feline, and caprine pyrogens.

Systems requiring in vitro activation are unfortunately quite complex. The addition

of human serum complicates the isolation of the active protein and the interpretation of cross-specific studies (37). It would be of great interest if activated human granulocytes obtained from fresh exudates could be shown to release a protein on incubation in saline that produces typical dose-responsive leukocytic pyrogen fevers in the rabbit. In view of all previous studies, any further cross-species studies with LP or EP should attempt to demonstrate clear dose-responsiveness and typical LP fever curves. To this end, temperature should be recorded at 5 min intervals or less for the 1st hr, and a full 3 hr curve should be presented to demonstrate uncomplicated defervescence.

Other recent developments in the study of the pathogenesis of fever include the description of tissue pyrogens in the rabbit (21), the description of endogenous pyrogen derived from mononuclear cells, both in rabbit and in man, as demonstrated by rabbit testing, (19, 20, 33), and the production of an endogenous pyrogen by human, but not by rabbit mononuclear cells on incubation with etiocholanolone (again demonstrated by rabbit testing) (34). The relationship of these newly recognized leukocytic, monocytic, and tissue pyrogens to each other, and to classic granulocytic pyrogen can not be resolved by fever testing alone. If and when a specific enzymatic assay or immunoassay becomes available for granulocytic pyrogen, the relationship of these various pyrogens may be clarified. Such techniques would also allow comparison of the various cell and tissue pyrogens with the variety of endogenous pyrogens found in the circulation during fevers of bacterial, viral, fungal, and parasitic infections, in hypersensitivity reactions, in agranulocytosis, in familial Mediterranean fever, malaria, and other febrile states not adequately explained by our current information.

## SUMMARY

Polymorphonuclear neutrophilic leukocytes of the dog, cat, and goat release leukocytic pyrogen under the same conditions as the heterophile polymorphonuclear leukocytes of the rabbit. The characteristics of the febrile response to an intravenous injection of homologous leukocytic pyrogen in all four species are very similar: a brisk monophasic fever reaching a peak between 30 and 50 min with smooth defervescence to the baseline by 3 hr. Shivering, which is not obvious in the rabbit, is noted in the dog, cat, and goat during the first 30 min. Quantitative differences in response reveal the cat to be the most sensitive of of these species to homologous leukocytic pyrogen, followed by the rabbit, dog, and goat.

The response to heterologous pyrogen is in most cases markedly diminished compared to that after equal doses of homologous protein, suggesting the operation of species specificity, although canine and feline pyrogen behaved very similarly in all tests. Species specificity of leukocytic pyrogen is probably related to amino acid substitutions in different species of a common mammalian protein effector molecule.

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