The Effect of Uremia on the Pyrogenic Response of Rabbits[1] (34244)

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It has been common clinical teaching that uremic patients do not develop as much fever with infections as do patients with normal renal function. However, there has been no thorough documentation of this observation in man or experimental animals. The present experiments compared the febrile response of normal and uremic rabbits to injections of rabbit leukocyte pyrogen (LP) and bacterial endotoxin. In addition, the ability of granulocytes from uremic animals to elaborate leukocyte pyrogen *in vitro* was evaluated.

Materials and Methods. Male albino rabbits weighing 2–3 kg were used in all experiments. Glassware was sterilized and rendered pyrogen-free by heating at 170° for 2.5 hr. Commercially available sterile, nonpyrogenic distilled water was used to prepare all solutions.

Rabbits were obtained in groups of 10 or 12. Uremia was induced in half of each group and the remainder served as controls. Animals were housed in pairs and allowed food and water *ad libitum*. The procedures employed for assaying pyrogens were essentially the same as those described previously (1). All animals were trained to the test situation, and on the day of challenge, temperatures were recorded every 15 min for 2 hr before injection of pyrogen. For purposes of subsequent calculations, the last recording was taken as the basal body temperature. As described previously (2), the febrile response was quantitated by means of the fever index (FI_{120}). The significance of observed differences between uremic and control animals was estimated according to the Student's *t* test.

Induction of uremia. Rabbits were made uremic by the intramuscular injection of 750 mg of the antibiotic cephaloridine (Lilly) on two successive days. In rabbits, this drug produces renal tubular necrosis, and within 24 hr, the animals became anorectic and oliguric (3). After 4 or 5 days, at the time of pyrogenic challenge, they appeared chronically ill and few animals survived longer than 1 week. Ten ml of blood was obtained from all animals at the time of pyrogenic challenge, and the concentration of blood urea nitrogen (BUN), calcium, and potassium was measured in the Mark-12 multichannel autoanalyzer.

In order to exclude the possibility that our observations were due to the effect of the drug and not uremia, bilateral ureteral ligation was performed in six rabbits. Three days later, blood was obtained for the same chemical determinations as in the cephaloridine-treated animals.

Response to leukocyte pyrogen. Leukocytes were obtained from peritoneal exudates according to procedures previously outlined (2). After the cells were washed 3 times in cold pyrogen-free saline, a final suspension containing 3.5×10^7 leukocytes/ml was incubated for 4 hr at 37°. The cells were removed by centrifugation, and the supernatant extract, which contained leukocyte pyrogen, was passed through a bacterial filter. Eighteen uremic and 18 normal rabbits received 1 ml of extract intravenously, and their febrile response was measured as outlined above.

Response to endotoxin. Twenty-one uremic and 23 normal animals were given 1.0 μg of *E. coli* 026:B6 lipopolysaccharide intravenously in a marginal ear vein. Rectal temperatures were recorded at 15-min intervals for 2 hr. In 5 uremic and 5 control

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TABLE I. BUN, Calcium, Potassium and Basal Body Temperature of Uremic and Control Rabbits.

	BUN (mg/100 ml)[a]	Calcium (mg/100 ml)[a]	Potassium (meq)[b]	Basal body temp (°)[a]
Uremic	179 ± 10.76	8.23 ± 0.31	6.35 ± 0.30	38.7 ± 0.13
Controls	12 ± 0.89	12.74 ± 0.45	5.00 ± 0.17	39.5 ± 0.03

[a] Differences statistically significant, $p < .001$.

[b] Many specimens contained hemolyzed blood; therefore, the Student's t test not performed.

animals, temperatures were recorded for 4 hr after injection of the endotoxin. The six rabbits with ligated ureters and a group of controls were challenged with endotoxin in the manner described above 3 days after surgery.

Production of leukocyte pyrogen. Exudates were induced in eight uremic and eight normal rabbits by instillation of 350–400 ml of a 0.1% glycogen–saline solution in the peritoneal cavity. The exudates were harvested 18–20 hr later and the leukocytes were washed three times in cold pyrogen-free saline. Differential counts were made to determine the proportion of polymorphonuclear leukocytes in the exudate, and a final leukocyte–saline suspension was adjusted to contain 3.5×10^7 cells/ml. Keeping the leukocytes from each animal separate, the suspensions were incubated with constant agitation for 4 hr at 37°. The cells were removed by centrifugation, and each extract was passed through a bacterial filter and stored at 0° until used. All samples were sterile when cultured under aerobic conditions on routine laboratory media.

A group of 16 rabbits which were tolerant to 0.1 μg/ml of endotoxin was used in the biological assay of these extracts. One ml of each sample was injected intravenously into 5 tolerant recipients and the febrile response was measured at 15-min intervals for 2 hr. For purposes of subsequent calculations, the average of the fever indices in all 5 recipients was taken as the FI_{120} of an extract.

Results. Induction of uremia. A detailed description of the model used for producing uremia will be published elsewhere. At the time of pyrogenic challenge, the BUN of animals which received cephaloridine ranged from 44 to 312 mg/100 ml with a mean of 179 mg/100 ml (Table I). Serum calcium values of treated animals ranged from 5.6 to 12.3 mg/100 ml, with a mean of 8.23 mg/100 ml. Serum potassium values ranged from 3.9 to 9.2 meq/liter with an average of 6.35 meq/liter in 28 animals. In the remainder, serum potassium was not measured because the flood was hemolyzed. The mean basal body temperature of 41 uremic and 41 normal rabbits was 38.7 cm^2 (SE 0.13) and 39.5 cm^2 (SE 0.03), respectively. This difference is highly significant with a p value of <0.001.

A regression line was plotted for the BUN as a function of basal body temperature. The correlation was highly significant as the basal body temperature varied inversely with the degree of axotemia (Fig. 1). However, there was considerable scatter of individual points along the regression line. A similar, but direct correlation was seen between

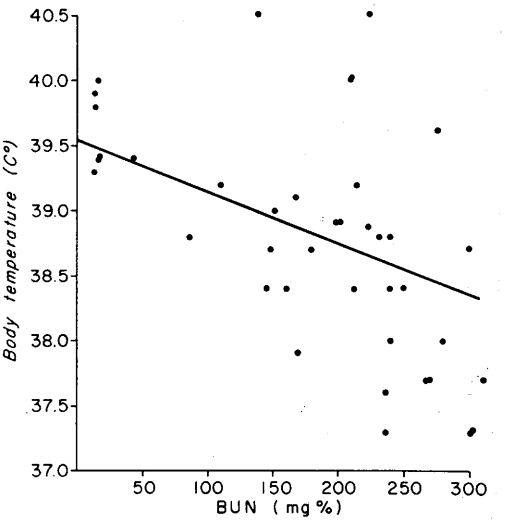

FIG. 1. The regression line shows the correlation between basal body temperature and BUN (mg/100 ml).

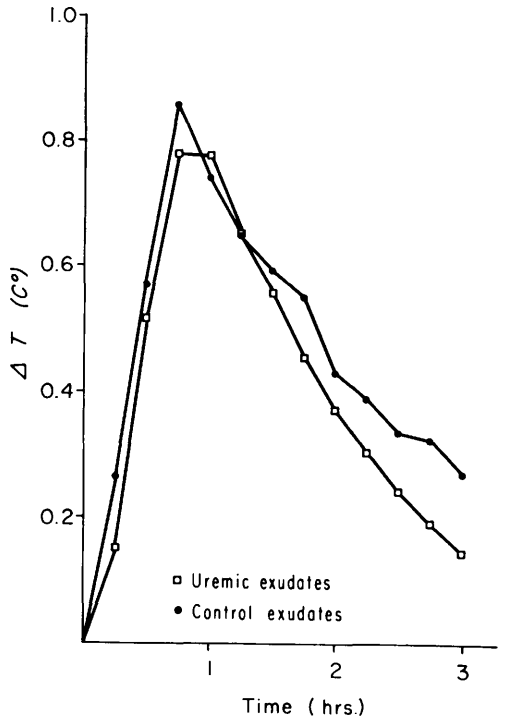

FIG. 2. The febrile response of uremic and control rabbits to leukocyte pyrogen.

serum calcium and basal body temperature.

Three days after undergoing bilateral ureteral ligation the mean BUN of eight rabbits was 172 mg/100 ml. The average serum calcium was 9.7 (SE 8.0) and the mean basal body temperature was 39.0 (SE 0.19). These results are similar to those recorded for the cephaloridine-treated group, but the number of animals is too small to permit meaningful statistical comparison.

Response to leukocyte pyrogen. Frequent individual variation in febrile response to administered LP was observed. However, the mean FI_{120} recorded for uremic rabbits was 41.6 cm^2 (SE 4.09) and for normal it was 45.1 cm^2 (SE 2.07). This difference is not statistically significant (Fig. 2).

Response to endotoxin. Both uremic and normal animals developed the usual febrile response to endotoxin (Fig. 3). The mean FI_{120} of the uremic rabbits was 65 cm^2 (SE 5.43) and it did not differ significantly from that of the normals, 70 cm^2 (SE 3.75). The manner by which uremia was induced did not alter the results; endotoxin also produced the same febrile response in normal rabbits and animals made uremic by bilateral ureteral ligation.

Production of leukocyte pyrogen. The total number of leukocytes in the peritoneal exudates from uremic and normal rabbits was approximately equal, and in both groups, 90% of the cells present were heterophils (rabbit polymorphonuclear leukocytes). When assayed in tolerant recipients, the pyrogenicity of the extracts from the peritoneal exudates of uremic animals did not differ significantly from those of normal rabbits. The mean of the FI_{120} of extracts from uremic animals was 37.9 (SE 2.25), while with extracts from normal rabbits it was 41.7 (SE 2.5).

Discussion. Numerous investigators have observed that uremic patients fail to develop as much fever as nonuremic patients during intercurrent infections (4–6). This relative lack of responsiveness to febrile stimuli has been attributed to the accumulation of toxic metabolites, because not infrequently, patients with acute renal failure develop a febrile response to infection after dialysis or spontaneous diuresis. Injection of urea (7), or an alcoholic extract of uremic plasma (8) produces hypothermia in laboratory animals, supporting the hypothesis that a toxic depres-

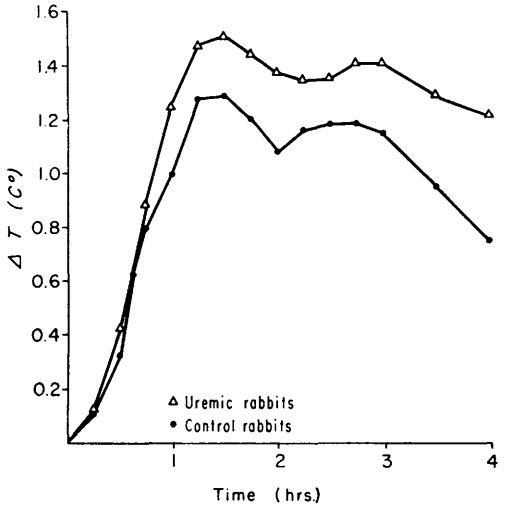

FIG. 3. The febrile response of uremic and control rabbits to 1.0 μg of bacterial endotoxin.

sion of thermal regulation occurs in uremia. It is not known, however, whether animals treated in this fashion have a normal response to pyrogenic stimuli.

Chronic renal failure alters many biochemical and physiological processes which may participate in the pathogenesis of fever, including protein synthesis (9), peripheral utilization of glucose (10) and central nervous system function (11), and an alteration in the febrile response might be expected in severe uremia. However, at least under the conditions of these experiments, uremia did not affect the response to a pyrogenic challenge.

These studies were intended to evaluate the febrile response at several different points. Firstly, the infusion of leukocytic pyrogen tests the thermoregulatory centers of the hypothalamus and the neuroeffector mechanisms which result in an increase in body temperature. Secondly, administration of bacterial endotoxin tests the animals' ability to elaborate endogenous pyrogen, which is biologically similar to leukocyte pyrogen (12). Finally, quantitating the number of leukocytes in peritoneal exudates, and measuring the pyrogenicity of extracts from these cells provides information about the effect of azotemia on the function of leukocytes and the formation of leukocyte pyrogen. If renal failure had influenced the synthesis of LP, some difference between the FI_{120} of leukocyte extracts from uremic and normal animals, should have been noted, because previous studies have shown that inhibitors of protein synthesis can alter the pyrogenicity of leukocyte extracts (2).

Although these results are valid for this experimental situation, they should be interpreted cautiously in relation to clinical observations, primarily because the response to endotoxin may not adequately reflect the physiological events by which fever is produced in naturally acquired infections. Detailed studies which are intended to evaluate the febrile response of uremic humans are now underway in this laboratory.

It is tempting to attribute the low basal temperature of uremic animals to some of the physiologic consequences of azotemia. However, many environmental factors such as position, degree of restraint, and amount of fur are known to influence the body temperature of rabbits (13). Many of the uremic animals had lost weight at the time of the pyrogenic challenge, and they were often too weak to sit in restraining cages unsupported. The observed correlations between the degree of azotemia, serum calcium, and the basal body temperature may also reflect only the extent of morbidity.

This study demonstrated the usefulness of cephaloridine as a nonsurgical means of producing acute renal failure in rabbits. Intramuscular administration of 200 mg/kg of this antibiotic uniformly results in injury to the middle third of the proximal convoluted tubule (unpublished observations). The drug is also reported to be nephrotoxic for other laboratory animals including monkey, rat, and mouse (3) and may be useful in a variety of experimental situations that require a uremic preparation.

Summary. In order to determine the effect of uremia on the febrile response to pyrogenic stimuli, normal rabbits and animals made uremic with cephaloridine were challenged with endotoxin and leukocyte pyrogen and their febrile response was recorded. In addition, the ability of granulocytes from untreated and uremic antimals to elaborate leukocyte pyrogen *in vitro* was evaluated. No difference was observed in the febrile response of normal or uremic animals to these pyrogens nor was any impairment noted in the ability of granulocytes from uremic animals to generate leukocyte pyrogen. A correlation between high levels of blood urea nitrogen, low values of serum calcium, and a lowered basal body temperature was found.

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