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Rabbit CRP Response to Endotoxin Administration: Dose-Response Relationship and Kinetics[1] (38260)

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Inflammation and tissue necrosis regularly result in formation of C-reactive protein (CRP). The mechanisms which mediate production of this protein are unknown (1). Correlative data suggest that the magnitude of the CRP response is related to the severity of inflammation (2), but a clear-cut quantitative relationship has not been demonstrated. In the current study, the CRP response was studied in rabbits injected with varying doses of endotoxin to define the relationship between severity of inflammation and CRP formation. In addition, the kinetics of CRP formation was investigated in these rabbits.

Materials and Methods. Male New Zealand White rabbits weighing about 5 lb were used throughout the study. In most experiments, rabbits with initially detectable serum CRP were employed. Immediately prior to each experiment, a preparation of endotoxin (*E. coli*/B; lipopolysaccharide 055:B5; lot No. 557630, Difco Labs) was dissolved in sterile pyrogen-free saline (Cutter Labs) to desired concentration and injected intravenously. Control animals were injected with pyrogen-free saline solution. In each instance a volume of 1 ml was employed.

0.5 ml blood samples were collected immediately prior to endotoxin injection and at intervals thereafter; each rabbit was bled a total of 14–16 times. In order to minimize stimulation of CRP production by handling and trauma, the following procedure was employed. Ear bleedings were carried out in or near the rabbit's cage. The ear was lightly wiped with an alcohol pad, the marginal ear vein punctured with a sterile microlancet (Becton, Dickinson and Co.) and blood allowed to enter a capillary tube (Rochester Scientific Co.). Serum was collected by a modification of the technique of Goldin and Kaplan (3), and stored in capillary tubes at −20°. CRP concentrations were determined in triplicate using the single radial immunodiffusion technique previously described (2); this method has been found to detect as little as 0.3 μg *N* CRP/ml quantitatively.

The magnitude of the CRP response was expressed in two ways: (a) the CRP Index (C.I.), measure of the height and duration of the CRP response, and (b) Δ CRP, the increase in serum CRP concentration from zero time to the peak value observed. To estimate the C.I., a compensating planimeter (Keuffel and Esser Co.) was used to measure the area above baseline values of the curve of serum CRP concentration plotted against time during the 72 hr following endotoxin injection. Results were expressed as Vernier units of the planimeter. Significance of correlations was determined employing the Student *t* test.

Results. Findings are shown in Table I. When plotted logarithmically, the CRP response was found to vary directly with the dose of endotoxin over the range of 0.1–10 μg. This relationship held true whether dose was plotted against CRP Index (Fig. 1) or against Δ CRP (Fig. 2). In each case correlation was significant ($P < 0.005$). With the lowest doses of endotoxin employed,

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TABLE I. CRP Response to Intravenous Endotoxin.

Endotoxin dose	No. of animals	Δ CRP concentration (μg N/ml)	CRP index (Vernier units)	Doubling time (hr)	Period of net production (hr)	Half-disappearance time (hr)
10 μg	5	5.8 ± 3.5	346 ± 183	7.5 ± 1.9	34 ± 8.9	12.7 ± 3.3
2.5 μg	7	12.4 ± 7.9	859 ± 593	5.0 ± 0.7	27.3 ± 4.9	11.4 ± 1.2
1.0 μg	6	2.5 ± 0.9	157 ± 65	5.9 ± 2.1	22 ± 4.9	12.4 ± 2.7
0.25 μg	6	4.2 ± 3.4	272 ± 243	9.0 ± 5.4	23.8 ± 1.0	14.2 ± 5.5
0.10 μg	5	2.2 ± 1.7	116 ± 63	8.9 ± 3.8	20.4 ± 6.8	16.1 ± 5.9
Significance of correlation with endotoxin dose		$P < 0.005$	$P < 0.005$	$0.10 > P > 0.05$	$P < 0.001$	$0.10 > P > 0.05$

0.01 μg and 0.025 μg, CRP response was minimal and did not exceed that seen in saline-injected controls.

The dynamics of CRP formation were estimated following endotoxin injection. In each case, following the injection of endotoxin, a plot of log CRP concentration against time revealed a phase of exponential increase in serum concentration (Fig. 3). Following a relatively brief period of slowing of CRP formation, a period of exponential decrease was noted. In most cases, serum CRP concentration had returned to baseline values within 72 hr after injection.

In 24 of the 29 rabbits studied, measurable amounts of CRP were present in serum at the time of endotoxin injection. In 17 of these 24 animals the period of exponential increase began immediately upon injection of endotoxin (Fig. 3), while in the other seven rabbits the onset of exponential increase was not immediate, a definite lag phase ranging from 4–12 hr (mean 6.1 hr) being noted (Fig. 4).

The duration of net CRP production, the period during which rate of CRP formation exceeded rate of catabolism, was estimated as the period between endotoxin injection and the highest observed serum CRP concentration. This period was directly related to the dose of endotoxin (Fig. 5). (The period of net CRP production in one animal who received 10 μg of endotoxin was particularly long [50 hr], apparently related to an extreme lag phase of 12 hr. Omission of this result from the data did not alter the correlation between the dose of endotoxin and the period of net CRP synthesis.) The doubling time of serum CRP concentration during the phase of exponential increase averaged 7.3 ± 1.8 hr for all animals studied. It tended to be slower in animals receiving smaller doses of endotoxin, but this relationship was not statistically significant ($0.10 > P > 0.05$).

During the phase of exponential decrease in serum CRP concentration a mean half disappearance time of 13.3 ± 1.9 hr was observed for the entire group of animals. Although half disappearance times were somewhat shorter in the animals receiving higher doses of endotoxin, a significant cor-

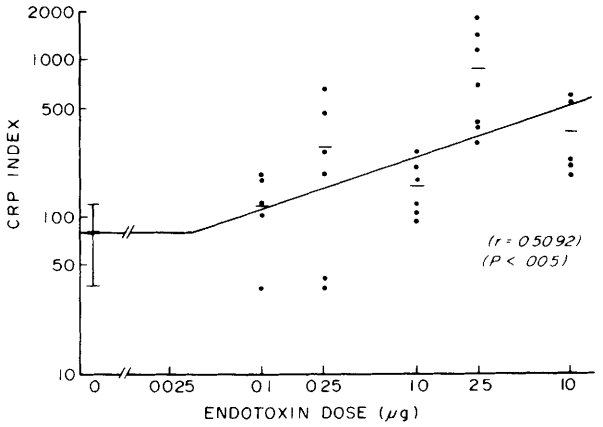

FIG. 1. CRP response, expressed as CRP Index (in Vernier units), to varying doses of intravenously administered endotoxin. Each dot indicates a single rabbit; mean values for each dose of endotoxin are indicated by a horizontal bar. The response to injection of saline, expressed as mean ± SD, is indicated above the zero value of the abscissa.

relation could not be demonstrated ($0.10 > P > 0.05$). Of note was a remarkably rapid half disappearance time of 4.8 hr in one animal who received 0.1 μg of endotoxin.

Discussion. We chose endotoxin as the inflammatory agent to elicit a CRP response since its intravenous administration has been shown to produce a graded, dose-related febrile response, one of the systemic manifestations of inflammation (4). The rate of formation of CRP was not determined precisely, but was estimated from the increase in serum concentration, on the assumption that the latter primarily reflected CRP production during the period of rapid formation. True rate of CRP synthesis can be no less than is estimated by this method, being greater to the extent that catabolism and diffusion into extravascular space affect the observed curves. The magnitude of the CRP response was found related to the dose of endotoxin administered. Higher doses of endotoxin caused a more prolonged period of net CRP production, indicating that the mediator stimulus leading to CRP formation was active for longer periods while a significantly more rapid rate of formation could not be shown. In studies of patients with myocardial infarction, a comparable correlation between intensity of inflammation and magnitude and duration of CRP formation was observed (5).

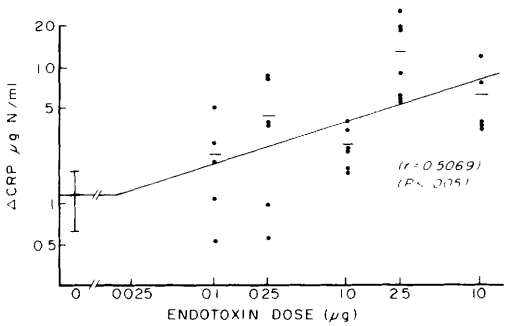

FIG. 2. CRP response, expressed as Δ CRP, the change in serum CRP concentration from zero time to peak value observed, following intravenous administration of various doses of endotoxin. See legend to Fig. 1 for details.

The dose-response relationship between endotoxin and CRP formation was found comparable to that between endotoxin and fever production (4). We were unfortunately unable to measure the temperature of the rabbits we studied, since insertion of a rectal probe resulted in a marked CRP response in these animals.

The exponential nature of the curve of CRP appearance and the rapidity of response are noteworthy. In some animals with doubling times of less than 5 hr, tenfold increases in CRP concentration were noted within 24 hr. The first order curve of appearance suggests either inflammation-induced rapid replication of hepatic cells capable of forming CRP (6), continuous recruitment of uncommitted cells to the task of CRP production, or exponential increase

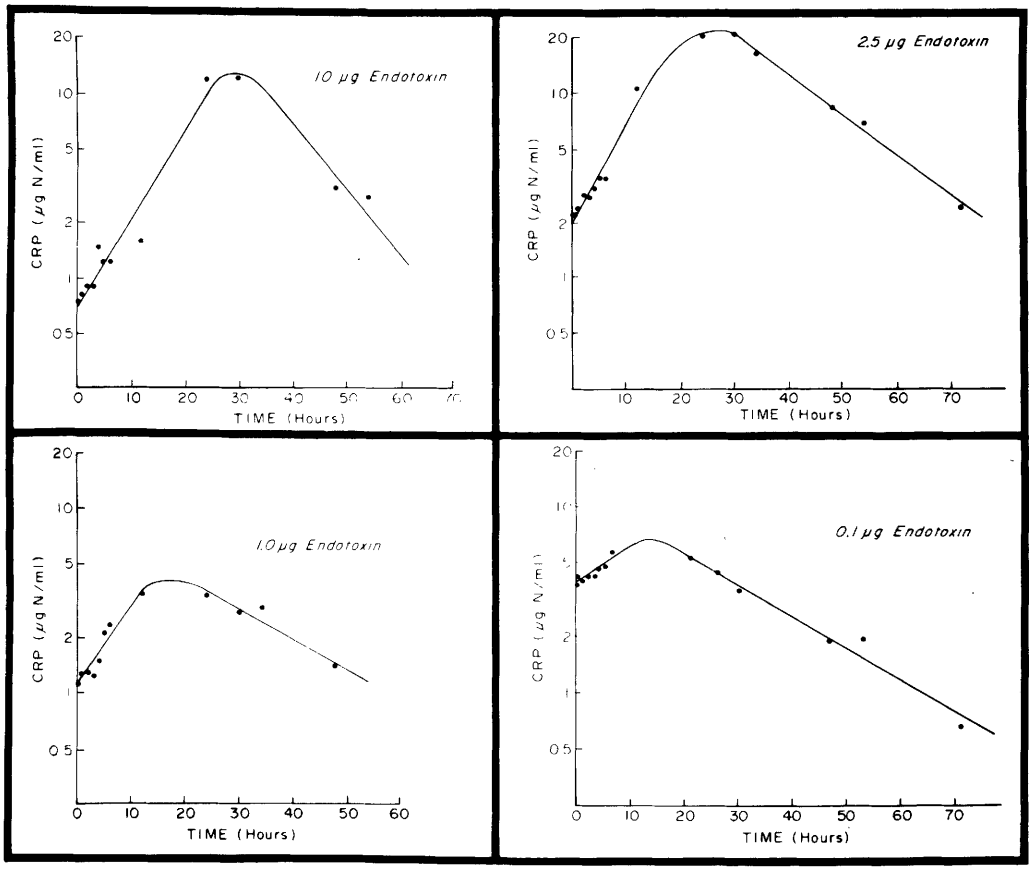

FIG. 3. Serum CRP concentration in four representative rabbits following intravenous injection of 10, 2.5, 1.0, and 0.1 μg of endotoxin.

in absolute rate of CRP formation per cell. It is not possible at this time to differentiate between these possibilities. Failure to find significant differences in doubling times between minimally and maximally stimulated animals suggests that the mediators inducing CRP production act in an all-or-none manner at a single and perhaps maximal rate, the amount of CRP formation depending on the duration of the mediating stimulus.

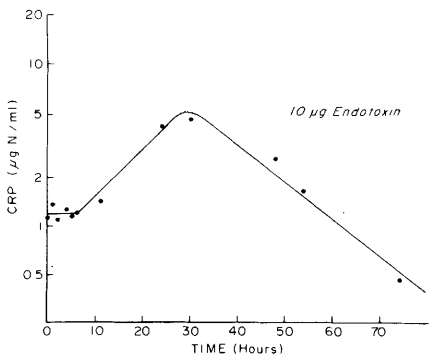

FIG. 4. CRP response to injection of 10 μg of endotoxin in a representative rabbit in whom a lag phase was noted prior to increase in serum CRP concentration.

A variable lag period of several hours before onset of CRP formation was noted in a minority of endotoxin injected rabbits, comparable to that noted in a minority of patients with acute myocardial infarction. The reasons for such a phenomenon are unclear. Most often, however, we found immediate onset of CRP production following stimulus, indicating that mediators leading to CRP formation ordinarily are produced and act very quickly.

The half-disappearance time observed in these studies is very short, compared to most plasma proteins, and represents a maximal true serum half-life. Some clotting factors

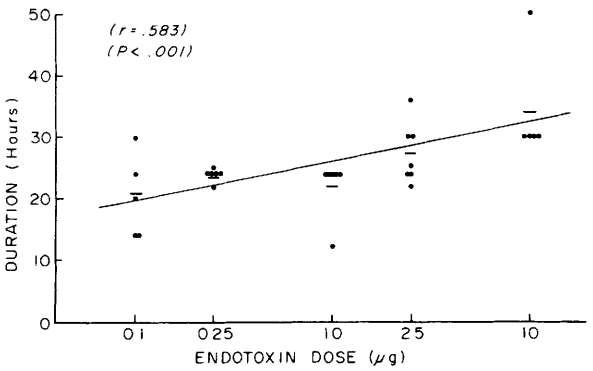

FIG. 5. Relationship between dose of endotoxin and duration of period of net CRP production. Each dot represents a single rabbit, mean values for each dose of endotoxin are indicated by a horizontal bar.

demonstrate comparable catabolic rates (7). It is reasonable to presume that the rapid disappearance of CRP results from rapid consumption in the course of playing an as yet unidentified functional role.

Summary. The duration of the period of net CRP production and the amount of CRP formed by rabbits in response to intravenous administration of endotoxin was directly related to the dose of endotoxin administered. Serum CRP concentration increased exponentially after endotoxin injection, with a mean doubling time of 7.3 ± 1.8 hr. In 71% of rabbits, the phase of exponential increase in CRP concentration appeared to begin immediately after injection, while an average lag period of 6 hr was noted in the remainder. The data suggests that mediators inducing CRP synthesis by the liver act in an all-or-none manner, the amount of CRP formation depending on the duration of the mediating stimulus.

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