Production of Fibrinogen Following an Endotoxin Injection (34599)

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Several studies have shown that injection of endotoxins causes the plasma fibrinogen level to rise (1–5). Fibrinogen is apparently synthesized in parenchymal cells in the liver (6) and secreted into the lymph (7, 8). The regulatory mechanism for the plasma fibrinogen level is not understood. Perfusion studies have indicated that the rate of synthesis depends upon the level of fibrinogen in the blood (9), but observations *in vivo* failed to show any relation between an elevated fibrinogen level and rate of synthesis (8).

In the present work it is shown that in rats the fibrinogen level rises abruptly starting about 8 hr after injecting a small dose of endotoxin subcutaneously. The rate of incorporation of glycine-^{14}C also increases markedly during the period from 8 to 16 hr after injection of the endotoxin. It is suggested that some step in the cellular reaction to inflammation initiates the increase in the plasma fibrinogen level.

Materials and Methods. Animals. Young adult female rats of the Carworth Wistar CFN strain were used. They weighed from 150 to 225 g. They were kept in individual cages to which they were accustomed for several days prior to the experiment.

Sampling. Blood samples were drawn from the heart under ether anesthesia. For dose response experiments, 0.5 ml of blood was mixed in the syringe with 0.05 ml of 0.1 *M* sodium citrate. For experiments requiring serial samples from the same rats 0.2 ml of blood was mixed with 0.3 ml of isotonic buffer. The buffer is prepared from MacIlvaine's buffer at pH 6.6 by diluting in the proportion of 3 vol of buffer to 1 vol of water. The packed cell volume is measured and this enables calculation of the extent of dilution of the plasma. Further details were published previously (10).

Fibrinogen assay. Plasma fibrinogen was determined either by a microgravimetric method (11) or by a heat-turbidity procedure. The heat-turbidity procedure involved mixing 0.1 ml of plasma or 0.2 ml of diluted plasma with 6.0 ml of MacIlvaine's buffer at pH 5.2. The mixture was heated at 55° for about 20 min. The resulting turbidity was measured as absorbance at a wavelength of 400 nm and converted to fibrinogen concentration by using a reference chart. This was a plot of heat turbidity vs. the concentration of fibrinogen as determined by a gravimetric reference procedure (12).

Endotoxins. Bacterial polysaccharides (Difco) from five bacteria were used separately for dose response experiments. The polysaccharides were from *Serratia marcescens, Escherichia coli, Staphylococcus aureus, Salmonella typhosa,* and *Salmonella typhimurium.*

The Difco polysaccharides were dissolved or suspended in physiological saline at a concentration of 300/μg/ml. Subcutaneous injections were made with the desired amount in 1.0 ml of saline.

Glycine-^{14}C incorporation. In order to study the rate of incorporation of labeled glycine into fibrinogen at different times after injection of polysaccharide, the first 24 hr were divided into three periods of 8 hr each. *Serratia marcescens* polysaccharide (30 μg/rat) was injected subcutaneously into all rats at the outset. Glycine-^{14}C labeled in the alpha position was injected directly into the heart blood at the beginning of each 8-hr period and a 2-ml sample of blood was drawn from the heart at the end of the period. The glycine solution injected was 0.20 ml of a

TABLE I. Fibrinogen Level in Rats Sampled 24 hr after Injection of Different Doses of Bacterial Polysaccharide.[a]

Dose[a] (μg/rat)	n	Fibrinogen (mg/100 ml)	SD
0	20	205	23.5
3	20	319	24.5
6	20	348	31.2
30	20	412	41.3
90	19	489	56.8
300	20	534	61.7

[a] Indicated dose was injected subcutaneously in 1 ml of saline.

solution of normal saline containing 0.22 mg of glycine and 2 μCi of ^{14}C. A control group received no polysaccharide but received glycine-^{14}C at the outset and was sampled at the end of the first 8 hr.

In one experiment (Table III) the plasma was diluted with 0.50 to 10 ml of saline containing 25 units of thrombin. The clots were collected on glass rods, washed in water, pressed on tared planchets, dried at 110°, and weighed on a microchemical balance. The time required for 1000 counts was measured. Background was 19 cpm.

In a second experiment the clots were hydrolyzed in hydrochloric acid and the glycine was isolated as the *N*-dinitrophenyl derivative according to the method of Krohl (13).

Results. Dose-response. Table I shows the effect of different doses of polysaccharide on the level of plasma fibrinogen 24 hr after subcutaneous injection. The fibrinogen level is proportional to the logarithm of the dose over the entire range. Dose response curves were run on each of the five endotoxins but no differences were found. The data were combined in Table I.

Time-response. A dose of 30 μg was selected as most suitable to study the change of fibrinogen with respect to time after injection of endotoxin. Table II shows the results combined from several experiments using *Serratia marcescens* endotoxin. The fibrinogen level increased rapidly from about 8 to 12 hr after subcutaneous injection of the toxin. From 0 to 8 hr and from about 15 to 24 hr the level did not change significantly. Beyond 24 hr the level slowly declines and reaches normal in 5 to 7 days. At a dose of endotoxin five times higher, the response is similar to that in Table II except that the level drops below normal at 2 hr after injection. It rises rapidly from 8 to 16 hr and continues to rise, more slowly, until 24 to 48 hr after injection.

The experiments in Table II involved fasting the rats for 3 days prior to injection of endotoxin and, in another, sampling with and without ether. The results showed no significant difference, so they were combined.

Incorporation of glycine-^{14}C. Tables III and IV show that the rate of incorporation of glycine-^{14}C was correlated with the rate of change in the plasma level of fibrinogen. During the first 8 hr after injection of polysaccharide there was little change in the fibrinogen level, and the rate of incorporation of glycine-^{14}C was the same as for a control period in rats not given polysaccharide. During the second 8 hr both the plasma level of fibrinogen and the incorporation of isotope increased markedly. During the third period, for 16 to 24 hr, when there is again little change in the plasma level, the rate of incorporation is again down into the range of the control.

Discussion. The results show that the fibrinogen level increases during a short period from 8 to 12 hr after injecting a small dose of bacterial polysaccharide subcutaneously in rats. Also, in all probability, the

TABLE II. Fibrinogen Level in Rats Sampled at Different Times after a Single Injection of Bacterial Polysaccharide.[a]

Hr after injection	n	Fibrinogen (mg/100 ml)	SD
0	46	211	30.4
2	25	199	32.2
5	30	231	47.6
8	24	230	36.5
9	29	274	35.9
12	17	389	53.3
15	29	397	59.1
16	24	411	51.7
24	43	422	63.8

[a] 30 μg/200 g/rat dissolved in 1 ml of saline and injected subcutaneously.

TABLE III. Extent of Incorporation of Glycine-^{14}C into Fibrinogen; Comparison of Different Periods after a Single Injection of Bacterial Polysaccharide.[a]

Rat group (hr)	n	Time of injection: Polysaccharide	Time of injection: Glycine-^{14}C[b]	Time of sampling[b]	Fibrinogen: mg/100 ml	Fibrinogen: Specific activity cpm/mg	Fibrinogen: Specific activity SD
Control	8	None	0	8	225	33.2	14.2
0–8	8	0	0	8	243	37.3	22.8
8–16	8	0	8	16	412	118.2	34.2
16–24	7	0	16	24	391	30.8	21.6

[a] 30 μg/200 g/rat dissolved in 1 ml of saline and injected subcutaneously.
[b] Hours after injection of polysaccharide.

increase represents synthesis of fibrinogen. It is true that the increased incorporation of glycine-^{14}C could result from a sharp decrease in the glycine pool size during the period that fibrinogen level is increasing, or it might result from another protein adhering to the clot and bearing the isotope, but these explanations appear unlikely. Koj and McFarlane (5) showed that injection of endotoxins in rabbits caused marked increase in the absolute synthetic rate of fibrinogen (over 400%) and only a moderate increase in that of albumin (60%) during the 24 hr following injection.

Several investigators have reported data showing that the fibrinogen level in other species also doesn't change for some hours after endotoxin injections. Rabbits given relatively heavy doses of endotoxin intravenously have no change in the fibrinogen level for over 6 hr, but it is increased twofold by 24 hr (3, 4, 14, 15). In humans, intramuscular injection of typhoid vaccine (1) or of endotoxin (16) caused a slight drop in the fibrinogen level during the first few hours, followed by an increase starting after the 12th but before the 24th hour.

The mechanism causing the increase in plasma fibrinogen probably involves a link in the chain of cellular events that occurs during the course of an inflammatory response (17–19). It has been found that sterile pus, from turpentine abscesses, contains a thermolabile factor that causes the plasma fibrinogen level to rise when it is injected intravenously or intramuscularly (20). The rise in plasma fibrinogen may result from the action of this factor after it is released to the circulation during the inflammatory reaction. Free fatty acids may control the rate of synthesis of fibrinogen. They are reported to stimulate fibrinogen production in liver slices by as much as 9.5-fold (21). Also, injection of endotoxin into rabbits causes the serum free-fatty-acid level to double within 2 hr and to stay elevated by around 50% for

TABLE IV. Extent of Incorporation of Glycine-^{14}C into Glycine Isolated from Fibrinogen[a]; Comparison of Different Periods after a Single Injection of Bacterial Polysaccharide.[b]

Rat group (hr)	n	Time of injection: Polysaccharide	Time of injection: Glycine-^{14}C	Time of sampling	Fibrinogen: mg/100 ml	Fibrinogen: Sp. act.[c] ± SD[c]
Control	16	None	0	8	196	0.35 0.043
0–8	16	0	0	8	223	0.37 0.071
8–16	16	0	8	16	410	0.60 0.200
16–24	16	J	16	24	414	0.29 0.142

[a] Glycine isolated as *N*-dinitrophenylglycine before counting.
[b] 30 μg/200 g/rat dissolved in 1 ml of saline and injected subcutaneously.
[c] Specific activity in cpm/μg of glycine ± SD.

about 2 days (22). However, it is difficult to reconcile the short initiation period with the considerably longer period before the fibrinogen level starts to rise.

Summary. Subcutaneous injection of bacterial endotoxins into rats causes elevation of the plasma fibrinogen level. Most of the increase occurs between 8 and 16 hr after injecting the endotoxin and incorporation of labeled glycine into fibrinogen is also highest during this time period.

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