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Rate of Clearance of Circulating Leukocytic Endogenous Mediator in the Rat (40912)

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Abstract. The rate of clearance of leukocytic endogenous mediator from the circulation of the rat was determined by measuring the rate of disappearance of activity from the plasma. Leukocytic endogenous mediator was injected iv into groups of rats and the plasma was periodically sampled for the activities that would lower plasma iron, increase plasma fibrinogen, or release bone marrow neutrophils. These activities were assayed by injecting the plasma into groups of normal rats. The $t_{1/2}$'s for the clearance of leukocytic endogenous mediator ranged between 6 and 10 min.

Phagocytic cells when properly activated release leukocytic endogenous mediator (LEM) which has been shown to alter metal metabolism (1–4), acute phase protein synthesis (5–8), and peripheral blood leukocytes (9, 10). The variety of biological manifestations produced suggests that this protein acts through several different sites (11). No information is currently available about the fate or rate of clearance of LEM from the circulation.

The most highly purified preparations of LEM are from rabbit peritoneal leukocytes (12). On Sephadex G-50 columns this LEM appears to have a molecular weight of 12,000 to 15,000 daltons and an isoelectric point at a pH of approximately 7.3. There is evidence that LEM is either identical or closely related to endogenous pyrogen (EP) (12, 13), and EP has been shown to have a short half-life in circulation (14). The site of removal of most of the circulating EP remains in doubt, since both the liver (14) and kidney (15) have been implicated.

In our experiments LEM was injected iv into rats and its rate of removal determined by periodically measuring the amount of activity remaining in the blood. Activity remaining was assayed by injecting the plasma into normal rats. Three different parameters were measured: fibrinogen concentration, plasma iron concentration, and the number of circulating neutrophils to assess remaining LEM activity. These assays indicated that the LEM activity had a half-life in the rats' blood of 6 to 10 min.

Materials and methods. Animals. New Zealand white rabbits weighing 3–4 kg were purchased locally. Female Holtzman-derived rats, 8 to 10 weeks old, were from our colony.

Preparation and partial purification of LEM. Crude LEM was obtained from leukocytes elicited into the rabbits' peritoneal cavity by the ip injection of 400–600 ml of 0.2% shellfish glycogen in saline (1, 9, 16). At 18 hr after injection, cells from the exudate were harvested, centrifuged, washed, and suspended in 0.15 *M* NaCl at a concentration of 1×10^8 cells/ml. The suspension was incubated at 37° for 2 hr, and the supernatant fluid containing the LEM was separated by centrifugation at 800*g* for 10 min. The crude LEM was further purified on a Sephadex G-50 column (16). The dose of LEM used was expressed in pyrogenic units, i.e., the amount required to raise the normal body temperature of a rabbit one degree (13, 16).

Measurement of biological activities. Heart blood, collected with a heparinized syringe from anesthetized rats, was used for total and differential leukocyte counts. Total leukocyte numbers were counted in a hemocytometer, and the percentage of neutrophils was determined from a 200-cell differential count of a Wright's stained smear (9, 16). Individual rats were used only once in the time-course experiments.

Plasma iron was determined on deproteinized samples by atomic absorption spectroscopy on a Model 403, Perkin–Elmer. Fibrinogen was determined by heat turbidity (17).

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Determining the rate of LEM disappearance from blood. Partially purified LEM (5 pyrogenic units/rat) was injected iv into groups of normal rats. At varying intervals after injection, heart blood was taken from the anesthetized rats. The heparinized plasma from each rat was separated by centrifugation and 1 ml/rat was injected iv into either two or three normal rats. Plasma iron and fibrinogen were measured 15 hr after injection, and the number of peripheral blood neutrophils at 1 hr.

Results. One milliliter of plasma from a rat, which 1 min previously had received an iv injection of 5 pyrogenic units of LEM, lowered the plasma iron of a normal rat about 150 μg/100 ml. Thirty minutes after the LEM injection, 1 ml of plasma only lowered plasma iron 36 μg/100 ml. The log plot of the disappearance of plasma iron-lowering activity is shown in Fig. 1. These data indicate a rapid destruction, inactivation, or removal of the iron-lowering activity from the plasma with a $t_{1/2}$ of 10 min.

Figure 2 shows the rate of disappearance of activity that promotes an increase in plasma fibrinogen from the blood. The fibrinogen concentration of normal rats averaged 185 mg/100 ml plasma. Injection of 1 ml of plasma, from a rat that 1 min previously had received an iv injection of 5 pyrogenic units of LEM, into normal rats increased fibrinogen concentration to 315 mg/100 ml plasma. Fifteen minutes after the LEM injection, 1 ml of donor plasma increased fibrinogen 42 mg/100 ml to an average value of 227 mg/100 ml plasma. The rate of disappearance of the fibrinogen activity had a $t_{1/2}$ of 9 min, which was close to the value obtained for plasma iron.

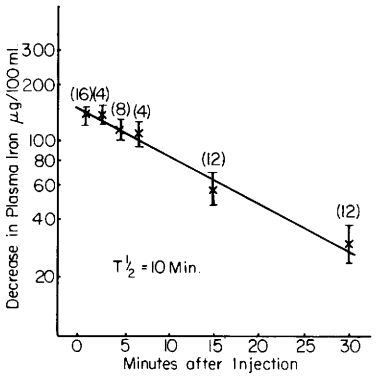

FIG. 1. Rate of disappearance from the plasma of the activity for decreasing plasma iron. Plasma was collected from rats at various intervals after they had received an iv injection of 5 pyrogenic units of LEM. Plasma from each donor rat was injected into either two or three normal rats with the total number used in parenthesis. Each recipient received 1 ml of plasma. Plasma iron was determined 15 hr later by atomic absorption spectrophotometer and the brackets show the standard error.

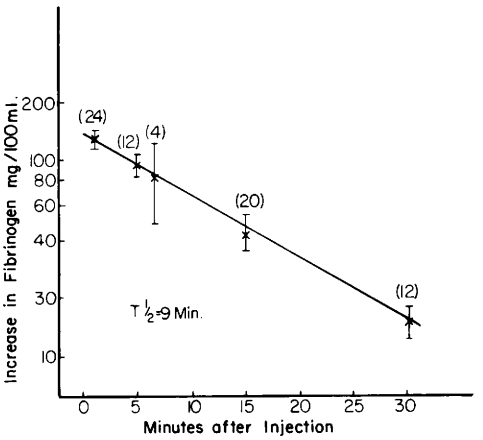

FIG. 2. Rate of disappearance from the plasma of the activity for increasing plasma fibrinogen concentration. Plasma from each donor rat (which received 5 pyrogenic units of LEM) was injected into either two or three normal rats with the total number shown in parenthesis. Each recipient received 1 ml of plasma. Fibrinogen concentration was measured 15 hr later by the heat turbidity method (17), and the standard error is shown by the brackets.

The average number of neutrophils found in a group of 19 normal rats was 1476 ± 148 neutrophils/mm^3 of blood. The number of neutrophils was increased by 4455 ± 454/mm^3 1 hr after a group of 9 rats received 1 ml of plasma from donors that 1 min before were injected iv with 5 pyrogenic units of LEM (Fig. 3). This activity disappeared rapidly from the plasma so that after 15 min 1 ml only increased blood neutrophils by 521 ± 297/mm^3 of blood.

Discussion. To have sufficient amounts of partially purified LEM, it was prepared from rabbit leukocytes and injected into rats. This may be contributing to the short $t_{1/2}$ of LEM in the plasma. It has been shown, however, that LEM prepared from

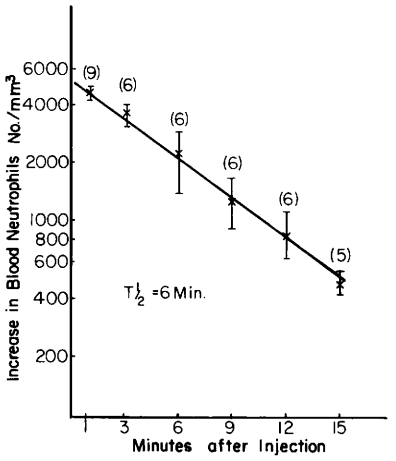

FIG. 3. Rate of disappearance from the plasma of the activity promoting neutrophil release from bone marrow. Each plasma donor had received an iv injection of 5 pyrogenic units of LEM. The plasma from each donor rat was injected into either two or three normal rats. Each recipient received 1 ml of plasma. Total circulating neutrophils were determined 1 hr later by total and differential counts upon blood obtained from the heart.

the rat or rabbit is equally active in the rat (1). It has also been shown that EP prepared from rabbit cells has a short half-life in the rabbit's blood stream (14).

The biological manifestations elicited after an injection of LEM resemble those seen during infection (18). There is, however, very little information available on the sites or mechanisms of action of LEM (11). During infection the biological effects can be observed for several days presumably due to a continuing production of LEM. After a single injection of LEM, however, the alterations that are produced return rapidly to normal, suggesting a rather brief half-life (1, 5, 7, 9). The similarities between the rates of removal of the activities for lowering plasma iron concentration ($t_{1/2}$ = 10 min), increasing fibrinogen concentration ($t_{1/2}$ = 9 min), and releasing marrow neutrophils ($t_{1/2}$ = 6 min) provide further evidence that they may all be mediated by the same protein (12).

Summary. The rate of clearance of leukocytic endogenous mediator from the circulation of the rat was determined by measuring the rate of disappearance of activity from the plasma. Leukocytic endogenous mediator was injected iv into groups of rats and the plasma was periodically sampled for the activities that would lower plasma iron, increase plasma fibrinogen, or release bone marrow neutrophils. These activities were assayed by injecting the plasma into groups of normal rats. The $t_{1/2}$'s for the clearance of leukocytic endogenous mediator ranged between 6 and 10 min.

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