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Effects of Leukocytic Endogenous Mediator on Hemopexin, Transferrin, and Liver Catalase (40118)

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Recent findings have shown that many of the acute phase reactions seen during inflammation or infection can also be produced by injection of leukocytic endogenous mediator (LEM). LEM is a low molecular weight protein that is released from stimulated granulocytes. Recent evidence suggests it is very similar or identical to leukocytic pyrogen (1). It has been found that LEM injection will increase the plasma levels of α_2-macrofetoprotein (2), ceruloplasmin (3), fibrinogen (4), haptoglobin (4), and C-reactive protein (5). All of these are glycoproteins that have an increased rate of synthesis in the liver during inflammation. Other proteins showing changes in response to acute stresses are transferrin, hemopexin, and liver catalase. Transferrin levels decrease after acute injury, which is the result of increased synthesis and an even greater change in the rate of catabolism (6). Likewise, liver catalase decreases following acute injury in this case due to an inhibition of synthesis (7). Less is known concerning the control of hemopexin levels after injury, but it has been suggested that in rats hemopexin acts as a typical acute phase protein (8). This suggestion was based on the finding of increased hemopexin synthesis in response to surgical trauma.

Since transferrin and liver catalase respond to acute stress quite differently from hemopexin and other acute phase proteins, their response to LEM may provide insight as to how LEM influences liver protein synthesis. The specificity of the LEM-induced changes in protein metabolism has not yet been fully established; however, should LEM injection reproduce all aspects of the acute phase response, it must be seriously considered as a possible mediator of the acute phase response. In this study we determined if LEM could simultaneously increase and decrease the synthesis rates of these different proteins as compared to plasma fibrinogen, which is known to increase (4).

Materials and methods. Animals. Holtzman derived rats were maintained at 21° with 12 hr of light and 12 hr of darkness. They were fed Rockland mouse and rat diet and water *ad libitum*. Healthy New Zealand white rabbits weighing 2–4 kg were used for LEM preparation.

Preparation of LEM. Crude LEM was prepared from rabbit peritoneal granulocytes (9) and partially purified by the butanol-methanol method of Rafter *et al.* (10). It was then filtered through a membrane which retained molecules of molecular weight >100,000 daltons (Amicon XM-100) and stored as a dry lyophilized powder. The dose of LEM injeted is expressed in terms of the number of leukocytes from which it was derived in the original preparation.

Determination of hemopexin, fibrinogen, transferrin, and liver catalase. Plasma levels of hemopexin were quantitated by radial immunodiffusion by the method of Mancini *et al.* (11). The monospecific antisera to rat hemopexin was generously donated by Dr. U. Muller-Eberhard, Scripps Clinic and Research Foundation, La Jolla, CA. Fibrinogen was determined on citrated plasma by the heat turbidity method of Wycoff (12). Transferrin was estimated from the total iron binding capacity of the plasma as determined by the method of Schade *et al.* (13). Liver catalase was measured by the method of Bonnichsen *et al.* (14). Statistical analysis was done with the use of Student's paired *t* test.

Results. The effects of injecting varying doses of LEM on hemopexin, fibrinogen, transferrin, and liver catalase in the rat are shown in Table I. The concentration of hemopexin and fibrinogen were increased at the smallest dose tested, and further increases were observed as the amount of injected LEM was increased. Decreases in transferrin and liver catalase occurred only at the highest dose tested. To increase the activity, we did further studies on these two proteins using

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TABLE I. EFFECT OF LEM DOSE ON HEMOPEXIN, FIBRINOGEN, TRANSFERRIN, AND LIVER CATALASE.[a]

Dose of LEM[b]	Hemopexin % of normal	Fibrinogen % of normal	Transferrin % of normal	Liver catalase % of normal
None	100 ± 5[c]	100 ± 12[c]	100 ± 3[c]	100 ± 3[c]
1×10^6	129 ± 11*	132 ± 5*	—	—
1×10^7	152 ± 8***	142 ± 7**	110 ± 2*	99 ± 4
1×10^8	210 ± 12***	197 ± 11***	93 ± 3	104 ± 3
5×10^8	—	—	85 ± 4**	90 ± 4*

[a] Measurements of transferrin were at 12 hr after LEM injections; the other proteins were determined at 24 hr after injection.

[b] Number of rabbit peritoneal granulocytes from which the leukocytic endogenous mediator (LEM) was derived. The partially purified LEM from 10^8 granulocytes had a protein content of approximately 180 μg.

[c] Mean ± SE of eight rats.

* Significantly different from normal $P < 0.05$.

** Significantly different from normal $P < 0.01$.

*** Significantly different from normal $P < 0.001$.

crude LEM before purification. We have no explanation for the apparent increase in transferrrin after injecting a low dose of LEM.

The plasma levels of hemopexin and fibrinogen at various times after LEM injection are shown in Table II. These levels were both significantly increased at 8 hr after injection and remained elevated for at least 24 hr.

Transferrin and liver catalase levels were also affected by a single injection of crude LEM (Table III). When the dose of LEM was expressed per granulocyte, the crude LEM was more active because of the substantial losses in activity during purification. Plasma transferrin levels were decreased significantly at 12 hr and then returned to normal values 24 hr after injection. Liver catalase followed a different time course in that it continued to decrease significantly throughout the 24-hr period after injection.

Further decreases in the level of both transferrin and liver catalase could be demonstrated after a series of injections of crude LEM. Injections of 10^8 LEM were made every 8 hr, and animals were sacrificed 5 hr following the final injection. As shown in Table IV, transferrin and liver catalase were significantly decreased following the fourth injection of LEM.

Discussion. These results confirm and extend earlier reports (1, 2, 4, 5) suggesting that injection of LEM induces many of the "acute phase" reactions that are typical of inflammation or infection. It should be noted that the hemopexin and fibrinogen levels are much more sensitive to LEM injection than are the levels of transferrin and liver catalase.

TABLE II. HEMOPEXIN AND FIBRINOGEN LEVELS IN THE PLASMA OF RATS AT VARYING TIMES AFTER INJECTING LEM.[a]

Time after ip injection (hr)	Hemopexin % of normal	Fibrinogen % of normal
0	100 ± 5[b]	100 ± 12[b]
4	118 ± 8*	109 ± 5
8	153 ± 9***	153 ± 10**
12	160 ± 6***	163 ± 6***
24	210 ± 12***	197 ± 11***

[a] Each rat received an ip injection of partially purified LEM prepared from 10^8 granulocytes dissolved in 1 ml of saline.

[b] Mean ± SE of eight rats.

* Significantly different from normal, $P < 0.05$.

** Significantly different from normal, $P < 0.005$.

*** Significantly different from normal, $P < 0.001$.

TABLE III. TRANSFERRIN AND LIVER CATALASE LEVELS AFTER A SINGLE INJECTION OF 5×10^8 CRUDE LEM.[a]

Hr after injection	No. of animals	Transferrin % of normal	Liver catalase % of normal
0	12	100 ± 2[b]	100 ± 4[b]
12	10	77 ± 1**	89 ± 3*
18	10	84 ± 3**	80 ± 4**
24	10	95 ± 3	75 ± 4**

[a] The dose of LEM is expressed as the number of rabbit peritoneal granulocytes from which the LEM was derived.

[b] Mean ± SE.

* Significantly different from normal, $P < 0.05$.

** Significantly different from normal, $P < 0.001$.

Differences in the amount of LEM required to cause other changes have also been noted. However, once this threshold dose of LEM was exceeded, their respective log-dose response curves were linear. Slope values for LEM-induced changes in fibrinogen levels

TABLE IV. TRANSFERRIN AND LIVER CATALASE LEVELS AFTER MULTIPLE INJECTIONS OF CRUDE LEM.

No. of injections[a]	No. of animals	Transferrin % of normal	Liver catalase % of normal
0	24	100 ± 4[b]	100 ± 3
1	10	93 ± 3	103 ± 3
4	8	70 ± 5*	55 ± 4*
7	10	83 ± 6**	40 ± 3*
10	10	65 ± 1*	49 ± 5*

[a] Each 8-hr injection contained LEM derived from 10^8 granulocytes.
[b] Mean ± SE.
* Significantly different from normal, $P < 0.001$.
** Significantly different from normal, $P < 0.05$.

and other acute phase reactions have been reported (1).

Previously it has been reported that plasma fibrinogen responds more quickly to injection of LEM than to injection of endotoxin (4). Comparison of the data in Table III with published data (15) reveals that transferrin also responds faster to LEM injection than to endotoxin injection. Not only is the greatest effect evident at an earlier time, but the return to normal is also much faster. This time course is compatible with the suggestion that LEM may be a mediator of the acute phase response.

Multiple injections of LEM appeared to be more effective than a single large dose. It seems likely during inflammation or infection that small amounts of LEM would be released from the granulocytes over a long period of time. Thus the multiple injections are assumed to more nearly approximate the *in vivo* release of LEM.

The finding that LEM injection could produce elevations in the plasma levels of hemopexin and fibrinogen and at the same time cause depression in the levels of transferrin or liver catalase indicates it has a specific function in the acute phase reaction. Previous investigations with an *in vitro* liver slice system have indicated that LEM does not act directly on the liver to produce its effect on fibrinogen synthesis but may act to release an intermediate which does stimulate *in vitro* fibrinogen synthesis (4). Further studies are needed to determine if the same chain of events is responsible for changes in the levels of other proteins.

Summary. Elevations in the plasma levels of hemopexin and fibrinogen were found following injection of leukocytic endogenous mediator (LEM) into rats. Liver catalase and plasma transferrin were decreased following injection of LEM. Greater amounts of LEM were required to depress liver catalase and plasma transferrin than were required to elevate hemopexin or fibrinogen. Multiple injections of LEM at 8-hr intervals were found to be more effective for depression of liver catalase and plasma transferrin than a single large dose of LEM.

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