A Comparison of the Effects of Rabbit Endogenous Pyrogen on the Body Temperature of the Rabbit and Lowering of Plasma Iron in the Rat (34423)

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The endogenous pyrogen that can be produced by rabbit granulocytes has been partially purified and found to be a small protein with a molecular weight of 10,000 to 20,000 (1–5). Some properties that help distinguish this protein from bacterial pyrogens are: induction of a brief monophasic fever of rapid onset; failure of tolerance to develop after repeated injections, full activity in animals tolerant or refractory to bacterial pyrogens; and inactivation by heat or by hydrolysis with trypsin or pepsin (1, 6–9). The endogenous pyrogen prepared from human or rabbit blood leukocytes, human mononuclear cells, and rabbit lung macrophages all seem to be similar in molecular weight and activity (5).

The crude extract from rabbit peritoneal leukocytes will, in addition to increasing the body temperature of the rabbit, cause the lowering of plasma iron (10) and body temperature (11) in the rat. The extract from rat peritoneal leukocytes will lower transport iron in the rat, but it has no effect on the body temperature of either the rat or rabbit (10). The object of the present investigation was to determine if different proteins were involved in the effects on temperature and plasma iron. To accomplish this, comparisons were made at various stages of purification of the effect of rabbit endogenous pyrogen on body temperature of the rabbit and plasma iron concentration in the rat.

Materials and Methods. The crude endogenous pyrogen was prepared from polymorphonuclear leukocytes induced in rabbits by intraperitoneal injection of 0.1% glycogen in saline as described by Rafter *et al.* (3). Partial purification was achieved by the modified butanol–methanol method (3). The partially purified endogneous pyrogen was eluted from a Sephadex G-200 column by the method Kozak *et al.* (4). Five separate polymorphonuclear leukocyte preparations, each representing exudates from 6–12 rabbits, were used. Each preparation was tested at various points during purification for biological activity in rats and rabbits. Before injecting these preparations, they were dialyzed against repeated changes of pyrogen-free distilled water. The ip injections in the rats were of this aqueous solution, but 9 mg/ml of NaCl was added to the iv injections given the rabbits. Pyrogenic activity was assayed in endotoxin-refractory rabbits (7, 9) by methods previously described (10). The lowering of plasma iron was measured by the method of Schade *et al.* (12) in 180–200-g female Holtzman rats 16 hr after an ip injection of the test dose. Tolerance to endotoxin was produced by giving 10 daily ip injections of 1 μg of endotoxin (10). Protein was measured by the Lowry modification (13) of the Folin procedure.

Results. Incubation of the endogenous pyrogen preparations in thioglycollate broth indicated no bacterial contamination. The following tests also affirmed the absence of bacterial pyrogens in these extracts: they produced a brief monophasic fever of rapid onset in rabbits made refractory to 5 μg of *E. coli* endotoxin; displayed full activity for lowering of plasma iron in rats tolerant to 1 μg of endotoxin; and were inactive in both rats and rabbits after heating or incubation with trypsin. The effects of both the crude and butanol–methanol extracts on plasma iron and body temperature are shown in Table I. The volumes injected were adjusted so that the yield from 1×10^8 rabbit leukocytes was given to each animal. After fractionation with butanol–methanol about 8% of the protein

TABLE I. Effect of Crude Endogenous Pyrogen and the Extract Partially Purified by the Modified Butanol–Methanol Method on Pyrogenic Activity in the Rabbit and Lowering of Plasma Iron in the Rat.

	Crude extract	Partially purified extract
No. of leukocytic cells used to prepare the extract injected	1×10^8	1×10^8
Protein injected (μg)	1320	106
Decrease in plasma iron of rats (μg/100 ml)	141 $\pm$ 7[a] (18)	121 $\pm$ 6[a] (16)
Increase in body temp of rabbits (°)	1.06 $\pm$ 0.07[a] (18)	0.99 $\pm$ 0.06[a] (16)

[a] Mean difference from controls receiving injections of either water or saline $\pm$ standard error for the number of animals shown in parentheses. The average control value for plasma iron in rats was 244 μg/100 ml and the control body temperature for rabbits was 39.2°.

remained, and it contained most of the pyrogenic activity and also the activity for lowering plasma iron in the rat. The average normal value for plasma iron concentration in these rats was 248$\pm$ 6μg/100 ml.

The partially purified protein was eluted from a Sephadex G-200 column and the elution pattern and biological activity are shown in Fig. 1. The protein peak in all 5 expts. was between 153 and 162 ml. The most activity for lowering plasma iron in rats and increasing body temperature in rabbits was found in this fraction. There was throughout

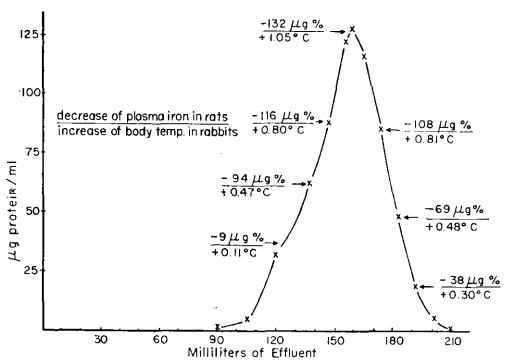

FIG. 1. Chromatogram of partially purified endogenous pyrogen on Sephadex G-200. The biological activity was measured at 7 different points by injecting 1 ml of the dialyzed eluate into rats and rabbits. The decrease in plasma iron from normal values in the rat is shown above the line and the fever produced in rabbits below the line. Three ml were collected in each tube and the 3 tubes (1 on either side of the point indicated) were combined for analysis. The results shown are averages for 5 expts.

the elution curve a good correspondence between the pyrogenic activity and the lowering of plasma iron. The amount of change in both activities seemed to be closely related to the μg of protein injected, except for the earliest fractions (up to about 125 ml) in which the specific activities were somewhat lower.

Discussion. The elution pattern from Sephadex G-200 corresponds quite well with those obtained by other investigators and indicates a protein with a molecular weight similar to ribonuclease (4, 5). Since the lowering of plasma iron in the rat accompanies the endogenous pyrogen so closely, it suggests that the same protein may be responsible. We had previously found that a crude leukocytic extract from the rabbit would affect body temperature and plasma iron in the rat and rabbit, whereas a similar extract from rat cells lowered plasma iron but had no effect on body temperature in either species (10). On the basis of this evidence we concluded that the endogenous pyrogen and the factor from leukocytes which decreases plasma iron concentration are not identical (10). The present results would indicate that the nonidentity may be applicable only to the protein from rat cells.

It was somewhat unexpected that the protein from leukocytes, which will lower plasma iron concentration, was similar to endogenous pyrogen, since the liver has been suggested as the site of action for lowering plasma iron (14) and the pyrogen probably

acts upon the hypothalamic thermoregulatory center (7).

Summary. The endogenous pyrogen from rabbit polymorphonuclear leukocytes was partially purified by butanol–methanol and elution from Sephadex G-200. This protein in addition to increasing the body temperature of the rabbit also lowered the plasma iron concentration in endotoxin-tolerant rats.

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