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Received July 19, 1963. P.S.E.B.M., 1963, v114.

## Relationship of Endogenous Pyrogen to Lysozyme.* (28682)

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Endogenous pyrogen (EP) is a material found in the blood stream of animals made febrile with bacterial endotoxins, certain colloids, viruses of the influenza group, following induction of experimental streptococcal, pneumococcal and staphylococcal infections and production of experimental hypersensitivity reactions(1). EP shares many of the biological properties of leukocytes or leukocytic extracts derived from inflammatory exudates, including ability to produce fever, and it has been assumed that EP is a product of leukocytes injured by a variety of pyrogenic stimuli(2).

EP can only be assayed by its ability to produce fever in a recipient of the same species. This method is relatively insensitive, cumbersome and expensive, imposes the limitation of species specificity, and involves the hazard of contamination by bacterial pyrogens which are ubiquitous in the environment. Because of these difficulties it seemed desirable to equate the biologic activity of EP with lysozyme, an enzyme which is easily measured by its action on a specific substrate, *Micrococcus lysodeikticus*. Lysozyme was chosen because of its high concentration in leukocytes which are presumed to be the major source of EP. In the present experiments alterations in concentration of lysozyme in serum were compared with changes in activity of EP under experimental conditions which are known to affect the quantity of EP in serum.

*Materials and methods.* Female mongrel dogs weighing 6-12 kg were housed unrestrained in air-conditioned rooms. Food and water were withheld on the day of experiment. Temperatures were measured rectally with clinical thermometers at 30-minute intervals for 4 to 6 hours. Endogenous pyrogen was prepared by intravenous injection of 3.0 ml typhoid vaccine containing approximately $10^9$ killed organisms into normal dogs. Animals were exsanguinated 120 minutes after injection of vaccine by femoral artery or cardiac puncture under pentobarbital anesthesia. Blood was permitted to clot, serum obtained by centrifugation, and cultured in thioglycollate broth. If found to be sterile, sera were stored at 4°C until used. Sera were never kept more than 2 weeks. EP was assayed by injecting 20 ml of serum into 3 normal recipient dogs. Animals used for pyrogen assay were permitted 3 hours for acclimatization and were not used if temperatures fluctuated more than 0.2°C. Fever curves were plotted on standard graph paper, the area beneath the curve for the first 2 hours after injection was measured by planimetry (Keuffel-Esser 423 M-9788) and the resulting area was defined as the fever index. Animals were made tolerant by daily injections of 3.0 ml typhoid vaccine for 9 days, and EP assayed from the same donor on day 1, 2, 5, and 9. Adrenal hormone used in all studies was Δ-1 hydrocortisone. Hypothermia was induced by immersing anesthetized animals in an ice bath and monitoring temperatures with a rectal thermister (Telethermometer, Yellow Springs Instrument Co.).

Lysozyme was determined prior to injection of endotoxin in each animal and on each sample of EP by the method of Kerby

* Supported by grants from U.S.P.H.S.

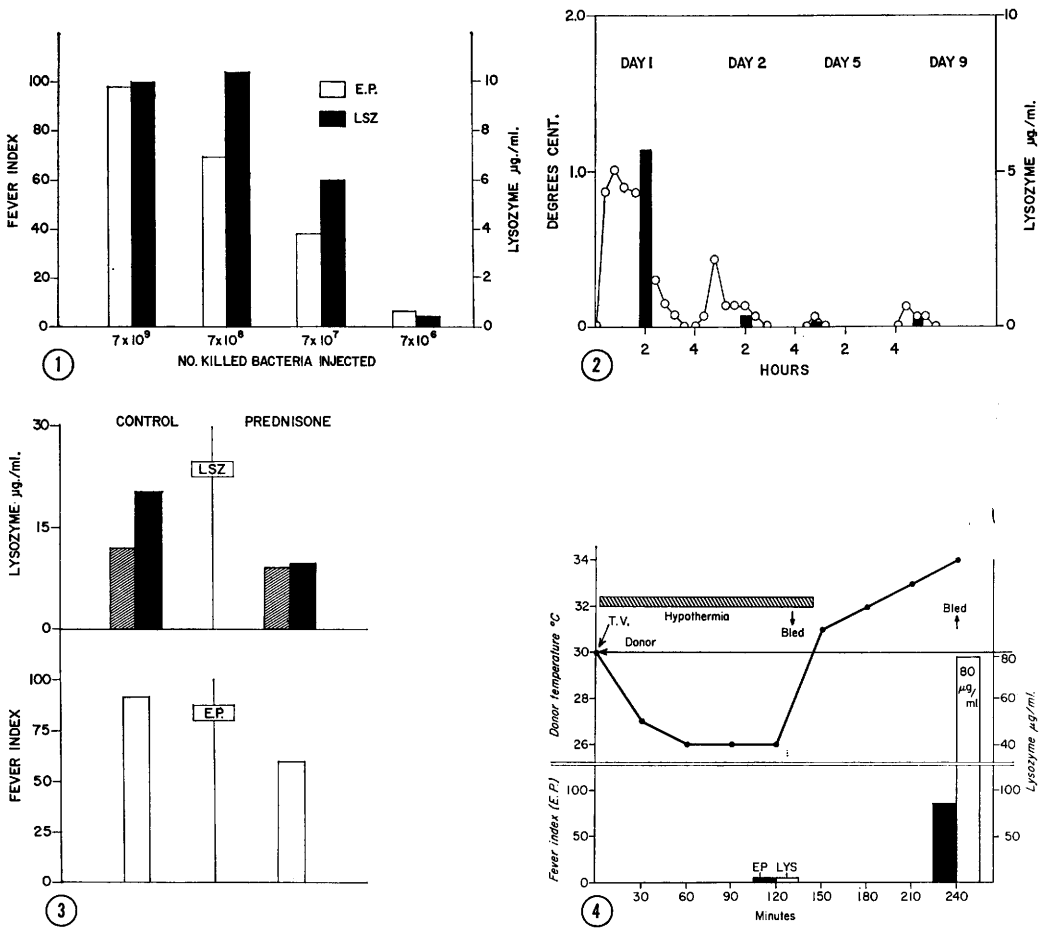


FIG. 1. Relationship between potency of endotoxin stimulus, EP and concentration of lysozyme.

FIG. 2. Progressive diminution of fever, EP and lysozyme with development of endotoxin tolerance.

FIG. 3. Effect of △-1 hydrocortisone on release of EP and rise in lysozyme in dogs given endotoxin.

FIG. 4. Effect of hypothermia on release of EP and serum lysozyme.

(4), using *Micrococcus lysodeikticus* as a substrate.

All needles, glassware, and instruments were sterilized at 170°C for 3 hours. All serum and other injectable agents were cultured in thioglycollate broth for 72 hours and discarded unless sterile.

*Experimental results.* Quantitative relationship between EP and lysozyme. Four groups of 3 dogs were injected with 10-fold concentrations of typhoid vaccine and bled 120 minutes later. Lysozyme concentration was determined prior to administration of endotoxin and on the sample EP. Fig. 1 demonstrates the direct relationship between dosage of typhoid vaccine and amount of EP released in response to the pyrogenic stimulus along with the change in lysozyme concentration in the serum.

Lysozyme and EP in tolerant animals. Repeated daily administration of endotoxin results in a progressive diminution in fever, a phenomenon termed "tolerance." Four dogs were made tolerant to endotoxin by daily injection of typhoid vaccine. Along with the expected decrease in fever, there was, with development of tolerance, a marked decrease

in EP paralleled by failure of lysozyme levels to rise (Fig. 2).

Relationship of tissue injury to release of EP and rise in serum lysozyme. Four animals were injected with 1.0 g of aminopyrine subcutaneously at time of injection of 3.0 ml of typhoid vaccine and again, one hour later. Aminopyrine completely suppressed the febrile response to endotoxin. The animals were bled 120 minutes after injection of typhoid vaccine and were permitted to recover. Three weeks later the experiment was performed in the same donors, omitting administration of aminopyrine. Although the animals receiving aminopyrine did not become febrile, their sera yielded similar amounts of EP and they developed an equivalent rise in lysozyme concentration compared to the response to endotoxin at a time when no antipyretic was given. This confirms previous experiments(5) that elaboration of EP in response to a pyrogenic stimulus is not affected by suppression of fever with aminopyrine. As might be expected, lysozyme concentration was also unaffected by vigorous antipyresis.

In another experiment, the antipyretic and antiinflammatory action of adrenal steroids on the release of EP and lysozyme was studied. Six animals were pretreated with 25 mg parenteral Δ-1 hydrocortisone per day for 48 hours. Following this they were challenged with 3.0 ml typhoid vaccine. Treated animals had less fever, released less EP and had only a small increment in serum lysozyme compared to controls challenged with endotoxin but not treated with adrenal hormones (Fig. 3). These studies again indicate a biologic parallel between EP and lysozyme, and suggest that steroids may exert an antipyretic effect by interfering with the inflammatory response.

In a third series of experiments, dogs were cooled to 26°C in an ice bath. When their temperatures had stabilized, the animals received 3.0 ml typhoid vaccine but by means of continued hypothermia, temperature was not permitted to increase. One hundred and twenty minutes after injection of typhoid vaccine, while dogs were still hypothermic, no detectable EP had appeared and serum lysozyme concentration had not risen. During the next 2-hour period, hypothermia was discontinued and the animals' temperatures rose from 26°C to 34°C. When bled 240 minutes after administration of typhoid vaccine, the animals' sera contained considerable amounts of EP and there had been a marked increase in serum lysozyme (Fig. 4). These experiments indicate that hypothermia delayed the injurious response of bacterial endotoxin and prevented release of EP and lysozyme while animals were hypothermic. As soon as hypothermia was eliminated, tissue injury took place which, in turn, was reflected by elaboration of EP and rise in lysozyme.

*Discussion.* These data demonstrate that concentration of EP and lysozyme parallel one another in animals given relatively large doses of bacterial endotoxin, made tolerant to the toxin, and subjected to endotoxic stimuli along with maneuvers aimed at altering the inflammatory response. The results are consistent with the hypothesis that both endogenous pyrogen and lysozyme are products of tissues injured by endotoxin, probably leukocytes. These cells have a high concentration of lysozyme and Kerby has demonstrated that they release lysozyme into the surrounding medium upon incubation with endotoxin *in vitro*(4). Furthermore, it has been shown that leukopenic animals challenged with endotoxin do not show a rise in lysozyme concentration in serum(6) and release no EP(7). Similarly, of many tissues tested, leukocytes have been the only ones demonstrated to possess pyrogenic activity (8). This evidence, then places the origin of both EP and lysozyme in the leukocyte.

Despite the many biological similarities between EP and lysozyme, there is presumptive evidence that these substances are not one and the same biochemically. Both are adsorbed to the colloid bentonite, resulting in abolition of pyrogenic as well as enzymatic activity. However, lysozyme is readily eluted from bentonite while EP is either firmly bound or inactivated by the colloid(9). In addition, other commonly used adsorbents, such as kaolin or charcoal, do not affect pyrogenic activity but adsorb lysozyme. These studies provide indirect proof that EP and

lysozyme differ biochemically. However, the relationship between these two substances will not be clarified definitely until both are characterized in precise chemical terms.

*Summary.* Endogenous pyrogen (EP) and lysozyme concentrations in serum paralleled one another when dogs were challenged with varying doses of endotoxin, were made tolerant to the action of the toxin, and were given endotoxin along with aminopyrine, adrenal steroids, and hypothermia. Despite these biologic parallels, there is no evidence that endogenous pyrogen and lysozyme are the same biochemical substance.

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Received July 19, 1963. P.S.E.B.M., 1963, v114.

## Selenium and Vitamin E Influence upon the *in vitro* Uptake of $Se^{75}$ By Ovine Blood Cells.* (28683)

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Incorporation of selenium into circulating blood cells following administration of radioselenium has been reported in swine(1), in the dog(2), in sheep(3) and in the chick(4). McConnell(2) has demonstrated that selenohemoglobin is produced following radioselenate administration.

The mechanism through which selenium enters the blood cell is not known, although the accumulation and retention of $Se^{75}$ following a single dose is dependent upon dietary selenium intake in the chick(4). While it is well documented that active transport mechanisms are present in the erythrocyte membrane for both sodium and potassium, it has not been determined from the *in vivo* experiments if such is the case for selenium.

These experiments were conducted to determine if the uptake of selenium by intact ovine blood cells *in vitro* would reflect the dietary intake of selenium. Sulfur, cesium, and zinc were studied to determine if the differences observed for selenium were specific for selenium or indicative of increased membrane permeability in the cells from the animals on the low selenium diets. In view of the observed interrelationships of selenium and Vit. E in prevention of certain pathological changes(5), the effects of dietary $\alpha$-tocopherol, both with and without supplemental selenium, were also investigated in this system.

*Methods and materials.* The basal ration used in these experiments is shown in Table I. The mineral mixture, essentially that of

TABLE I. Percentage Composition of Basal Diet.

| | | | |
|---|---|---|---|
| Cellulose* | 30.0 | Stripped lard† | 2.0 |
| Corn starch | 29.7 | Choline chloride | .1 |
| Cerelose | 29.7 | Ground plastic‡ | 1.0 |
| Urea | 4.0 | Mineral mixture§ | 3.5 |

* Solka-floc, BW-40. Brown Co., New York.
† Molecular distillation, gift of Distillation Prod. Ind., Rochester, N. Y.
‡ Polyethylene, M-700. E. I. du Pont de Nemours Co., Wilmington, Del.
§ Percent: $CaHPO_4$, 48.84; $K_2CO_3$, 31.55; $MgSO_4$, 10.75; NaCl, 7.43; $FeSO_4$, .91; $Na_2B_4O_7$, .19; $ZnSO_4$, .14; $MnSO_4$, .10; KI, .03; $CuCO_3$, .02; $MoO_3$, .0008; $CoCl_2$, .0003.

* Published with permission of the Director, Univ. of Tennessee Agri. Exp. Station, Knoxville.

† Operated by Tennessee Agri. Exp. Station for U. S. Atomic Energy Commission under Contract No. AT-40-1-GEN-242.