duce oral potency. 6β fluorination made both parent steroids inactive by either route of administration. P-DHP and A-DHP have longer duration of activity than progesterone, moderate progestational effects still being present 25 days and 20 days, respectively, following a single 10 mg intramuscular injection. P-DHP is devoid of inherent androgenic, anti-androgenic, estrogenic, anti-DCA or corticoid activities.

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Absorption of Endogenous Pyrogens by Bentonite.* (26297)

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Animals made febrile with bacterial endotoxins and myxoviruses have a substance in the circulation which is capable of producing fever when passively transferred to normal and tolerant animals(1,2). This substance which has been termed endogenous pyrogen (E.P.), is biologically distinct from the initial pyrogenic stimulus(3). E.P. has also been demonstrated in serum of animals with experimental pneumococcal(4), streptococcal(4), and staphylococcal infections(5), and in sensitized animals challenged with tuberculoprotein(6). The E.P. found in serum shares many of the biological properties of peritoneal exudates or leukocytic extracts which are also capable of producing fever (7). On this basis it has been suggested that E.P. is a product of injury to leukocytes by pyrogenic stimuli(3).

The chemical constitution of E.P. has not been determined, although recent evidence suggests that it is a protein(8). It is also not known whether endogenous pyrogens found following a variety of thermogenic stimuli are biochemically identical. One of the difficulties in answering these questions is that identification of E.P. rests solely in its ability to produce fever in animals. It seemed reasonable therefore to attempt to correlate the appearance of E.P. with the ap-

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pearance of some other product of injury to leukocytes. It has been demonstrated that lysozyme mirrors injury to leukocytes(9), and, like endogenous pyrogen, is found in increased concentration in the circulation of rabbits given bacterial endotoxins(10).

In comparing similarities between E.P. and lysozyme it was found that both substances can be absorbed from serum by bentonite and endogenous pyrogens from several sources were absorbed by this substance.

Methods. Male rabbits of mixed breed weighing 3-6 kg were housed in air-conditioned rooms and permitted rabbit pellets *ad libitum.* To record temperatures, animals were confined to metal stalls with a loosely-fitting collar. Temperatures were measured rectally by thermocouples (Telethermometer, Yellow Springs Instrument Co.).

Endogenous pyrogens were prepared by intravenous injection of 1.0 ml typhoid vaccine containing approximately 10^9 killed organisms into normal rabbits. Animals were exsanguinated 120 minutes after injection of vaccine. Blood was permitted to clot, serum cleared by centrifugation, and cultured in thioglycollate broth. If found to be sterile, sera from several animals were pooled and stored at 4°C until used. A second group of donors was given 4.0 ml of an allantoic fluid suspension of Newcastle disease virus (NDV) with hemagglutinin titer of 1:1024. Virus suspension regularly produced fever in donors. The animals were bled 4 hours later and the sera processed as described above. In a third group of animals peritoneal exudates were prepared by intraperitoneal instillation of 500 ml of 0.85% NaCl. Four hours later peritoneal fluid was aspirated by gentle suction. Most exudates contained 10^6-10^7 leukocytes per ml. Cells were sedimented by centrifugation and resuspended in sterile 0.85% NaCl. After mixing for 24 hours, cells were removed and cell-free saline suspensions used in experiments. These suspensions were invariably pyrogenic in normal recipient rabbits.

Bentonite was baked at 170°C for 3 hours to eliminate contamination with bacterial pyrogens. One gram of this material was thoroughly mixed with 10.0 ml of serum E.P. or exudate and left at 4°C overnight. The bentonite was removed by centrifugation at 4°C and 5.0 ml of absorbed serum or exudate was injected into a normal recipient; the remainder was used for culture and determination of lysozyme activity. Two rabbits were used for each experiment; each received 5.0 ml of E.P. before and after absorption by bentonite. One animal was given unabsorbed E.P. on the first day and absorbed E.P. on the following day; the procedure was reversed in a second animal.

Lysozyme was determined on most aliquots of E.P. before and after contact with bentonite by the method of Kerby(9) using *Micrococcus lysodeikticus* as substrate.

In one series of experiments, dogs were given 5.0 ml of typhoid vaccine intravenously and bled 5 minutes and 120 minutes later. Sera were processed as described above and 20.0 ml aliquots were tested in normal dogs both before and after absorption with bentonite.

Precautions to avoid contamination by bacterial pyrogens of all solutions, needles and glassware were taken. Fever indices were calculated as described previously(7).

Results. Fig. 1 illustrates the mean fever curves of 6 pairs of rabbits given serum E.P. produced by administration of endotoxin or NDV, and leukocytic pyrogen derived from peritoneal exudates. Each type of E.P. produced a brisk febrile response in normal rabbits, and each contained from 6.5 to 11.0 μg/ml of lysozyme. Following mixing of bentonite with serum or exudate, in ratio of 100 mg/ml, pyrogenic activity was almost completely eliminated and lysozyme content reduced to less than 1 μg/ml.

Reduction of pyrogenic activity was directly proportional to amount of bentonite mixed with E.P. as was demonstrated by dividing 100 ml of serum endogenous pyrogen into 5 equal parts, each of which was absorbed with 1, 10, 50, and 100 mg or no bentonite per ml E.P. respectively. 5.0 ml aliquots from each pool were tested in 3 rabbits. The results are summarized in Table I. Pyrogenic activity was almost totally abolished by absorption with 50 mg bentonite per ml E.P. On the other hand, complete

absorption of lysozyme was accomplished with 1.0 mg. In another experiment designed to assay rapidity of absorption, it was found that pyrogenicity was totally removed within 15 minutes after contact between E.P. and bentonite. Other commonly used adsorbents such as kaolin and charcoal failed to affect pyrogenic activity but absorbed lysozyme.

The effect of absorption with bentonite on the fever-producing effect of bacterial endotoxin is summarized in Table II. In this experiment the pyrogenicity of typhoid vaccine mixed with saline and serum, before and after exposure to bentonite was tested in each of 3 rabbits. Serum obtained from donor animals 5 minutes (endotoxin-in-serum) and 120 minutes (E.P.) following intravenous injection of 1.0 ml of typhoid vaccine was similarly tested. Although there was slight diminution in thermogenic activity of endotoxin-serum mixtures and endotoxin-in-serum (5 min serum) following contact with bentonite, only 120 minute-serum (E.P.) was rendered completely non-pyrogenic. Adsorption to bentonite also failed to affect the pyrogenicity of NDV.

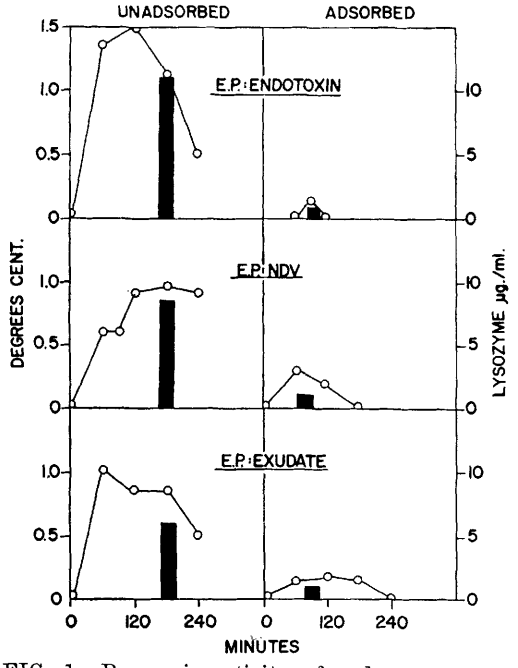

FIG. 1. Pyrogenic activity of endogenous pyrogens produced by administration of endotoxin and NDV, or in leukocytic exudates before and after absorption with bentonite. Lysozyme activity decreased concomitantly.

TABLE I. Quantitative Relationship between Amount of Bentonite Used to Absorb E.P. from Serum and Reduction in Pyrogenic and Lysozyme Activity.

Bentonite, mg/ml E.P.	% pyrogenic activity remaining	% lysozyme activity remaining
—	100	100
1.0	55	—
10	36	—
50	3.5	—
100	1.9	—

TABLE II. Fever Indices of Endotoxin, Endotoxin in Serum, and Serum E.P. before and after Absorption by Bentonite.

Material tested	Fever index	
	Unabsorbed	Absorbed
1.0 ml TV + 4.0 ml N. saline	22.8	30.0
1.0 " " + 4.0 " NRS	42.5	30.9
5 ml 5′ serum	20.8	13.6
5 " 120′ "	15.8	3.0

In another experiment, E.P. obtained from dogs following administration of typhoid vaccine was tested in these animals before and after admixture with bentonite. The results were similar in every respect to those obtained in rabbits and will not be detailed.

Discussion. The observation that several biologic characteristics of lysozyme and E.P., such as reduction of pyrogenic and lysogenic activity by bentonite, tend to parallel one another suggests that lysozyme may be a useful, easily-measured indicator of E.P. which does not entail use of animals. Parallel measurements of both substances need to be carried out under a variety of conditions to determine more precisely the relationship between lysozyme and E.P. Since lysozyme has been found to be an index of injury to leukocytes, the present findings support the idea expressed by Atkins and Wood (1), that E.P. appears when granulocytes are damaged by a variety of pyrogenic stimuli.

A number of silicate minerals under proper conditions of pH and solvent composition are known to adsorb charged particles. Bentonite has predominantly exposed negative charges at certain points and hence positively charged particles are adsorbed to it(11).

The probability that E.P. carries a net positive charge is consistent with its failure to be adsorbed to DEAE, an anionic exchange cellulose(8). The ability of bentonite to inactivate endogenous pyrogens produced by a variety of methods suggests that these fever-producing substances carry the same charge. However, the precise chemical structure of several types of E.P. remains to be determined. It is also of interest that the pyrogenic activity of typhoid vaccine was not altered by bentonite, adding further support to the idea that endogenous pyrogen is different from exogenously administered pyrogens. On the other hand, serum-endotoxin mixtures were inactivated to a limited extent, perhaps because the augmenting effect of normal serum on endotoxin was partially reduced(12).

The mechanism governing the action of bentonite upon E.P. is not clear. It is most probable that the silicate merely absorbs E.P. from serum or leukocytic extracts but attempts to elute E.P. from bentonite have met only with sporadic success. In the majority of instances eluates from bentonite have been non-pyrogenic. These negative results may be due to failure to break the bond between E.P. and bentonite, inactivation of E.P. during adsorption or elution, or elution of only part of the E.P. absorbed. The concentration of the E.P. in these partially eluted samples was probably too small to elicit a pyrogenic response. Experiments are in progress to increase the yield of these eluates.

Summary. Endogenous pyrogen (E.P.) in serum from animals given endotoxin or Newcastle disease virus and in leukocytic exudates was inactivated by absorption with bentonite. Concentration of lysozyme in serum and exudates paralleled that of E.P. The pyrogenicity of typhoid vaccine and NDV was not affected by absorption with bentonite.

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