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D A Bass

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Research Article

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Behavior of Eosinophil Leukocytes in Acute Inflammation

I. LACK OF DEPENDENCE ON ADRENAL FUNCTION

DAVID A. BASS

From the Nuffield Department of Clinical Medicine, University of Oxford, Oxford, England

ABSTRACT Acute infection is accompanied by a characteristic reduction in circulating eosinophils. This study examined the generally held assumption that the eosinopenia of infection is a manifestation of adrenal stimulation. Trichinosis, *Escherichia coli* pyelonephritis, and early subcutaneous pneumococcal abscess were used as experimental infections of limited severity. Trichinosis is associated with eosinophilia, but pyelonephritis and pneumococcal infection produce eosinopenia. An assay for serum corticosterone was developed that is sufficiently sensitive to be performed with the small volumes of blood obtained sequentially from individual mice. The corticosterone response to trichinosis fits the stereotyped reaction previously reported for several other bacterial, viral, and rickettsial infections. The peak concentration of corticosterone in serum from mice with trichinosis was approximately twice normal and occurred at the onset of clinical illness. Serum corticosterone levels gradually declined to the normal range over the next several days. *E. coli* pyelonephritis produced a similar adrenal response, although the peak serum corticosterone caused by pyelonephritis was less than the serum corticosterone occurring during the first peak of eosinophilia during trichinosis. Infection of a subcutaneous air pouch with pneumococci produced eosinopenia within 6 h after inoculation, but there was no rise in serum corticosterone during the first 12 h of the pneumococcal infection. In addition, the eosinopenic response produced by a 12-h pneumococcal abscess occurred in mice adrenalectomized 1–4 days before infection with pneumococci. The eosinopenia of acute infection cannot be ascribed to adrenal stimulation.

Dr. Bass was a recipient of a grant from the Commonwealth Fund. His present address is the Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Tex. 75235.

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INTRODUCTION

Acute infections are typically accompanied by a marked reduction in the number of circulating eosinophil leucocytes. This characteristic eosinopenia of acute infection was first described by Zappert in 1893 (1) and was utilized during the first quarter of this century as a useful diagnostic sign (2). In 1934 Spink (3) demonstrated that infection with staphylococci, tubercle bacilli, or trypanosomes could obliterate the eosinophilia in guinea pigs with experimental trichinosis. After the observation that eosinopenia is part of the normal response to stress (4) and may be mediated by endocrine mechanisms (5), it was assumed that the eosinopenia of acute infection is a secondary response to "stress" caused by the infection (6). By this hypothesis, acute infection stimulates the release of adrenal glucocorticoids, which then cause the decrease in the number of circulating eosinophils. This hypothesis for the mechanism of eosinopenia has never been proven. The observations that "stressful" systemic infections such as trichinosis may be accompanied by an eosinophilia, whereas relatively localized acute infections like pyelonephritis can induce eosinopenia, suggest that eosinophils may show specific responses to inflammation that cannot be explained by a single mechanism related to stress. Clarification of the mechanisms that control eosinopenia in acute infections could provide insight into the physiological roles of eosinophils and might explain the dramatic differences in the responses of eosinophils and neutrophils to acute infections.

Techniques developed in this laboratory during the past several years facilitated such studies by defining an experimental animal model which provided a well controlled, predictable eosinophilia. Such a model was necessary for the accurate quantitation of eosinopenic responses. Immediately before the beginning of this study, Morgan and Beeson (7) demonstrated that the

mouse rendered eosinophilic with trichinosis developed an acute eosinopenic response to a superimposed acute bacterial infection such as pyelonephritis.

The present investigation examines the role of adrenal corticosteroids in producing the characteristic eosinopenia of acute infection. An assay for corticosterone was developed that is sufficiently sensitive to permit determinations of corticosterone in serial serum specimens from individual mice. The serum corticosterone responses to trichinosis, with its associated eosinophilia, and to pyelonephritis or pneumococcal abscess, with their associated eosinopenia, were compared. Acute pneumococcal infection in adrenalectomized animals was shown to induce a characteristic eosinopenic response. Thus, these observations indicate that the eosinopenia of acute infection is independent of adrenal stimulation.

METHODS

Animals. C3H/mg mice were obtained in a LAC 4 (specific pathogen-free) condition from the Medical Research Council Laboratory Animal Center, Carshalton, Surrey, and Scientific Agribusiness Consultants, International, Ltd., Braintree, Essex. Two separate animal rooms were used. On arrival from suppliers, mice were placed in a small room reserved for these animals. It had its own air supply from outside and contained a filter cage rack (Carworth Europe, Alconbury, Huntingdon) which supplied a laminar air flow filtered to 5 μm designed to minimize cross contamination between cages. Mice were kept in North Kent Plastic Cages (Dartford, Kent) with a maximum of six per cage. Cages were sterilized at high temperatures, the sawdust was autoclaved, and sterile small animal diet (Spillers Ltd., Gainsborough, Lincolnshire) was given with tap water ad libitum. When mice with eosinophilia were required, they were given trichinosis by oral infestation utilizing moderate aseptic conditions (gloves, sterilization of equipment) during the procedure. Such animals were then housed in the lower half of the filter cage rack for the 3 wk required for trichinosis to progress to the desired stage. 2 days before further experimentation, the mice were transferred to a second room with controlled light and temperature, as already described (8).

Eosinophil counts. Peripheral blood samples were obtained from the retro-orbital venous plexus under light ether anesthesia. Methods of eosinophil counting have already been described (8).

Experimental trichinosis. The Culbertson stain of *Trichinella spiralis,* originally obtained from the London School of Hygiene and Tropical Medicine, was maintained by serial reinfestation of rats by oral inoculation of muscle stage larvae. Muscle stage larvae were obtained by digestion of infected muscle tissue with artificial gastric juice. Details of methods for obtaining and quantitating muscle stage larvae have already been described (8). To produce experimental trichinosis in mice, the animal was anesthetized lightly, and the stomach was intubated with a blunted no. 11 needle, through which 200 larvae (20 larvae/g body weight) were injected. Preliminary studies showed that a significant eosinophilia was obtained with an infecting dose between 150 and 1,000 larvae per mouse with relatively little alteration in magnitude of the resultant eosinophil response.

Pneumococcal infection. Streptococcus pneumoniae type 3 was obtained from the Central Public Health Laboratory, Colindale, London (batch no. 3NR2). To maintain virulence, the organism was passed through a mouse every 2 mo by i.p. infection followed by culture of the heart blood after 18 h. As required, the organism was cultured in Todd-Hewitt broth enriched with 10% defibrinated sheep blood and incubated for 9 h at 37°C with gentle agitation.

When administered by the i.v. or i.p. route, the pneumococcus produced a fulminant infection and death within 6–24 h depending on inoculum size. However, Smith and Wood (9) had shown a prolongation of the normal course of infection if a heavily encapsulated type 3 pneumococcus was introduced in a large inoculum into a large subcutaneous air pouch in the rat. Adaptation of this model to the mouse provided an infection of abrupt onset lasting for 24–30 h after bacterial inoculation (with only mild systemic stress after 12–14 h, the usual time of study). To produce the air pouch, 2.5 ml of air was injected subcutaneously over the thoracic spine. This required anesthesia but could be performed in advance if the experiment demanded absence of stress at the time of inoculation. An inoculum of 0.1 ml containing 2×10^8 organisms was injected into the pouch with a no. 25 needle. Control animals were given an air pouch into which 0.1 ml sterile saline was injected. The condition of the organism was important. A type 1 pneumococcus or an older culture of type 3 (with resultant partial loss of capsule) resulted in rapid development of cellulitis and death without appreciable local exudate formation.

Acute pyelonephritis. Acute unilateral pyelonephritis due to *Escherichia coli* was induced by a modification of the method of Freedman and Beeson (10). The organism was obtained from a patient with acute pyelonephritis, and identification was by colony formation and fermentation of sugars. When needed, this was grown overnight on MacConkey's agar, after which one colony was inoculated into beef heart infusion broth and incubated for 4 h; an inoculum of 0.05 ml of a 1:10 dilution of this culture, containing 10^5 organisms, was used. The mouse was anesthetized with sodium pentobarbital, and the ureter was ligated after approach through an anterior abdominal incision. Immediately after ligation, the bacterial inoculum was injected into the renal medulla with a no. 27 needle. Sham operated control animals underwent laparotomy and a similar ureteral dissection but no ligation or renal injection. The peritoneum was then closed with 4/0 silk suture, and the skin was repaired with 9-mm stainless steel clips. 2 days later, an inoculated obstructed kidney would be distended with purulent urine containing more than 10^6 bacteria per ml. Occasionally cortical abscesses could be seen at this stage; they were invariably present by the 4th day. The infection remained confined to the kidneys.

Adrenalectomy. After sodium pentobarbital anesthesia, a single skin incision was made over the spine, and each adrenal was approached through small incisions through the flank lateral to the paraspinal muscles and caudal to the 12th rib. Through this opening, each adrenal was approached from above, the vascular pedicle of the gland was gripped with curved forceps, and the adrenal was plucked out with its capsule intact. Incisions through tht flank muscles were closed with a single 4/0 silk suture, and the skin incision was repaired with steel clips. Adequacy of adrenalectomy was checked by assaying for corticosterone at the end of each experiment. The assay used could detect a serum level of 1 μg/100 ml, and animals with detectable serum levels were discarded. After adrenalectomy, the mice were

maintained on 5% glucose and 0.9% saline as drinking water with very few later deaths.

Corticosterone assay. A modification of the competitive protein binding method originated by Murphy (11) provided an assay of sufficient sensitivity to allow sequential sampling from mice. Corticosterone-1α,2α-^{3}H (Radiochemical Centre, Amersham) was obtained with a specific activity of 44 Ci/mM; this was first diluted 1:10 with ethanol as a stock solution and further diluted with aqueous buffer as needed. Aqueous buffer used as diluent through the assay was 0.05 M, pH 7.4 Sørenson phosphate buffer. Soda glass test tubes 10 × 30 mm (Gallenkamp, London) were used throughout. Preliminary studies showed that these adsorbed approximately 6% of the steroids in the sample size being studied but that the amount was uniform and hence would be compensated for in the standard samples. Microliter quantities were measured with glass constriction micropipettes.

Extraction of corticosterone from serum samples was accomplished by solvent partition. The serum sample (10 or 25 μl) was diluted with buffer to make the aqueous phase 75 μl. Dichloromethane (Koch-Light Labs, Colnbrook, Buckinghamshire), 1.5 ml, was added, and the solutions were mixed on a vortex agitator for 30 s and allowed to stand for 10 min. They were then held at −20°C until the aqueous layer would freeze in a ring around the test tube; the dichloromethane could then be decanted through the frozen aqueous layer by simply inverting the tube. This organic phase was dried at 45°C under an airstream. It was then ready for redissolving in 200 μl of buffer or a previously standardized mixture of binding protein and tritiated corticosterone. Preliminary experiments demonstrated that this one-step procedure provided 83% extraction of serum corticosterone with a coefficient of variation of 3.2%.

To determine an appropriate binding protein, preliminary experiments comparing serum from monkey, rat, rabbit, or humans during estrogen treatment ("human pill serum") demonstrated that the human pill serum had the greatest binding of corticosterone of the samples tested. Such human pill serum was also available in sufficient quantity to allow preparation of a large standardized mixture of binding protein and tritiated corticosterone which was frozen in small portions at −20°C and used as required, thus minimizing interassay variability. For this purpose, serum was obtained from a woman on estrogen therapy. The serum was treated with charcoal (Norit A purified decolorizing, Fisher Scientific Co., Pittsburgh, Pa.) at 50 mg/ml for 30 min at room temperature to adsorb all endogenous steroids present. The charcoal was removed by centrifugation, and the serum was sterilized by 0.45 μm filtration (Millipore Ltd., Wembley, Middlesex). For use, the serum was diluted with buffer to 1:64–1:512 dilution as required; tritiated corticosterone was added to provide 50 pg/200 μl concentration. This mixture was held at 4°C until needed. It was then added in 200 μl quantities to the tubes containing the dried samples (after dichloromethane extraction). This sample-binding protein-tritiated corticosterone mixture was then equilibrated for 1 h at 4°C before separation of the protein-bound and unbound fractions.

Removal of the unbound corticosterone was achieved by adsorption onto dextran-coated charcoal (12). The adsorbent was prepared by mixing a suspension of 1.25 mg/ml of charcoal (Norit A purified decolorizing) and 0.125 mg/ml dextran T70 (Pharmacia Fine Chemicals, Uppsala, Sweden) in the Sørenson buffer. This was prepared in 200-ml volumes which were agitated by a magnetic stirrer throughout the assay. In rapid succession, 200 μl portions of the suspension were added to a maximum of 20 tubes, each containing 200 μl of the previously equilibrated sample-binding protein-tritiated corticosterone mixture. Exactly 5 min after addition to the first tube (and 4.5 min after the last), the tubes were centrifuged at 1,500 *g* for 10 min. Of the supernatant, 250 μl were pipetted into 7 ml scintillant. Scintillant was prepared by dissolving 104 g naphthalene, 6.5 g PPO (2,5-diphenyloxazole, Koch-Light Labs), and 0.13 g POPOP (1,4-bis-[2-(5-phenyloxazoyl)]benzene, Koch-Light Labs) in 300 ml methanol, 500 ml dioxane, and 500 ml toluene (British Drug Houses). The sample was counted for 10 min on a Beckman LS 200 liquid scintillation counter (Beckman RIIS Ltd., Glenrothes, Fife, Scotland). Maximal counting error was 2%. In each series of tubes, three were included that were treated with 200 μl of buffer rather than charcoal-dextran, thus retaining all of the labeled steroid in the supernate and providing a "100% bound" standard.

A standard curve was determined during each assay. A weighed amount of unlabeled analytical grade corticosterone (Koch-Light Labs) was dissolved in a small volume of ethanol and diluted in buffer (final ethanol concentration less than 2%) to provide samples of 0.25–64 ng corticosterone per 25 μl. These were then treated in exactly the same manner and at the same time as unknown samples. Standard curves so obtained depended on the concentration of binding protein used. The 1:64 dilution provided a sensitivity of 1.1 ng of corticosterone and a precision of 10-12% from 1.1 to 20 ng of corticosterone; the 1:512 dilution provided a sensitivity of 0.25 ng and a precision of 15–20% from 0.25 to 8 ng as determined by the method of Braunsberg and James (13).

In initial experiments mouse blood samples were obtained by decapitation. This was performed in a manner such that the time interval between walking into the room and the end of the sampling period was less than two min. This method was cumbersome and prevented the possibility of sequential sampling. Anesthesia with sodium pentobarbital was known to produce a slight rise in serum corticosteroids followed by a fall back to resting levels by 30 min (14). It thus offered the possibility of obtaining true resting values on anesthetized mice after waiting the appropriate interval. With this method, mice were anesthetized with i.p. sodium pentobarbital; the injection was performed with a limit of 15 s handling, and the mouse was kept anesthetized for 30 min before sampling. Samples of 100 μl were obtained from the retro-orbital venous plexus by use of 100 μl calibrated tubes of 1.7 mm external diameter (DisPo micropipettes, Scientific Products Div., American Hospital Supply Corp., Evanston, Ill.). The tube was then tilted to allow the blood to flow toward the opposite end, which was then flame-sealed with a directional propane torch, avoiding overheating the blood held away from the end of the tube. The sample was then centrifuged on a microhematocrit centrifuge, the tube was broken at the cell-serum junction, and the serum was expelled onto paraffin. Serum corticosterone values obtained by the methods of decapitation or by the method of sodium pentobarbital anesthesia showed no significant difference either in normal mice or in mice stimulated by 0.1 IU ACTH given i.v. 20 min before sampling.

Statistics. The variability of the eosinophil response of different batches of mice necessitated internal controls on all experiments. As the majority of experiments involved a transient change in eosinophil levels, significance was tested by comparing the two or more populations on the day of the experiment. Whenever possible, eosinophil counts were done

the day before and the mice divided into the necessary number of groups according to their pre-experiment eosinophil levels, thus assuring equivalent groups. Mice with unusually aberrant eosinophil levels on the day preceding the experiment were discarded. In comparison of populations, the nonparametric Wilcoxon Rank Sum Test was used.

RESULTS

Normal values. Eosinophil counts were consistently low in resting C3H/mg mice. In 90 mice, the normal eosinophil count was 65±60/mm³ (mean ± 2 SD) with a range of 5–125/mm³. In 12 mice, the normal concentration of corticosterone was 6.66±4.30 μg/100 ml with a range of 2.36–10.96 μg/100 ml in samples of serum from blood collected between 9:00 and 11:00 a.m.

Response of eosinophils to trichinosis in mice. The responses produced by oral infestation with 200 *Trichinella spiralis* larvae per 20 g mouse were determined in 83 animals over a period of 4 mo (Fig. 1). Two separate peaks of eosinophilia were observed. Although not seen in the C3H mice used by Morgan and Beeson (7), a similar curve with two peaks has been observed in Ash/XP and CBA/H strains in this laboratory (unpublished data). The first peak of eosinophilia occurs at the time of first larval migration through the intestinal submucosa into the bloodstream and is associated with a local eosinophilia in intestinal lymphatic tissue (Peyer's patches) (15). This response is not prevented by depletion of thymus-derived lymphocytes (16), and the mechanism of eosinophilia at this phase of trichinosis is not known. At the time of the second peak of eosinophilia, trichinella larvae are well encased in a granulomatous inflammatory process in striated muscle. The second peak of eosinophilia is inhibited by lymphocyte depletion (16) and appears to be analogous to the lymphocyte-mediated eosinophilia of trichinosis in rats (17).

As long as the mice were free of other infections, the timing of the second peak of eosinophilia was predictable. The height of the peak showed little variation within each group of mice, although it varied considerably between different groups. Therefore, the height of this peak was determined in control animals in each experiment.

Changes in serum corticosterone levels in response trichinosis. Blood samples from mice with trichinosis were obtained under sodium pentobarbital anesthesia. In individual mice, a maximum of three blood samples was collected with a minimum interval of 4 days between sampling. Samples from 5 to 10 different mice were obtained on each day after infestation with *Trichinella spiralis.* Serum corticosterone concentrations showed a sharp rise to a peak of 16.2±1.2 μg/100 ml (mean ± SEM) on day 11, the time of larval migration, and declined gradually over the following 10 days to normal levels (Fig. 2). Therefore, serum corticosterone concentrations were elevated during the first

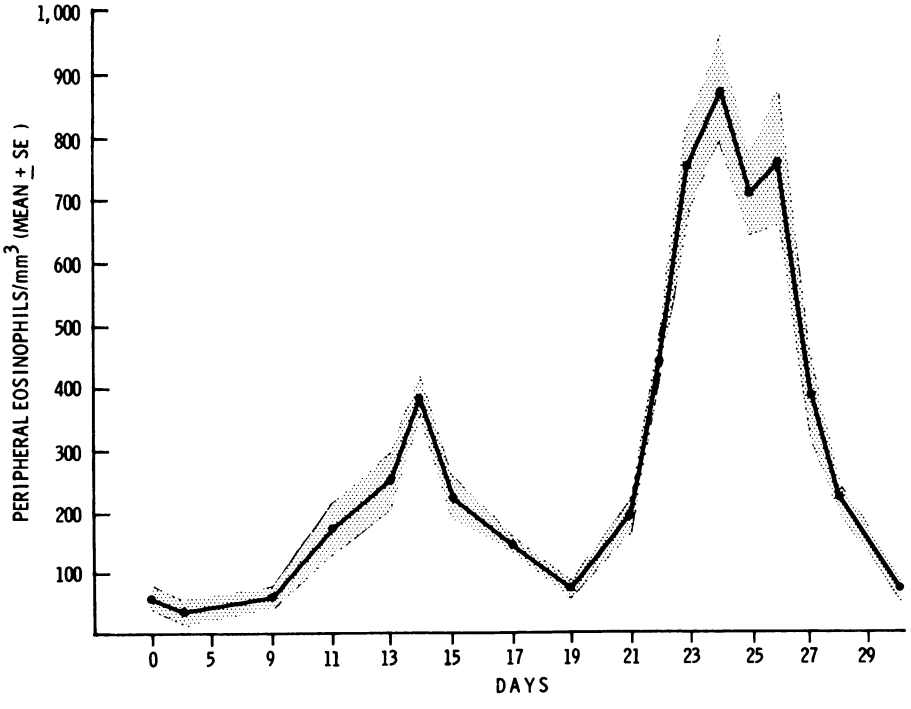

FIGURE 1 Eosinophil counts in C3H/mg mice with trichinosis (mean±SEM). For days 0–19 and 28–30, each point is the mean of 12 mice, and for days 21–27, 83 mice.

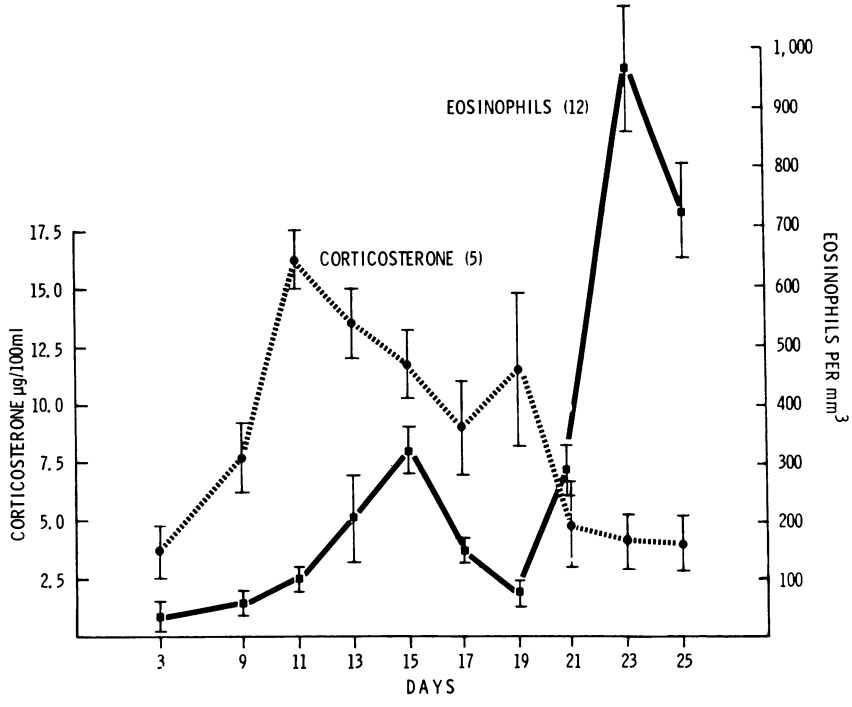

FIGURE 2 Serum corticosterone and eosinophil counts in mice with trichinosis (mean±SEM).

peak of the blood eosinophil response but had returned to normal levels at the time of the second peak of eosinophilia.

Use of the eosinophilic mouse to study induced eosinopenia. To obtain predictable responses of eosinophils to various stimuli, the mice must be free of adventitious infections. Pathogen-free animals used in these studies have a low and consistent eosinophil count in the resting state. With such low eosinophil counts, difficulties in obtaining statistically significant changes prohibited a study of stimuli which induced further eosinophil leucopenia, but the predictable, reproducible eosinophilia of trichinosis provided a higher base line for studies of induced eosinopenia. Because the high second peak of eosinophilia was remarkably constant in its timing, potentially eosinopenic infections were initiated at that time. The procedure utilized was to determine eosinophil counts on day 22 of trichinosis to verify that the eosinophilic response was underway. Animals were then separated into equivalent groups, and the inflammatory stimuli were applied. Experimental and control groups were compared on days 23 and 24 of trichinosis at the expected height of eosinophila.

Response of eosinophil counts to acute pyelonephritis. Earlier studies in our laboratory (7) had indicated that acute pyelonephritis induced by the method of Guze and Beeson (18) is an acute, limited, reproducible infection of sufficient severity to cause an eosinopenic response. For the present study, acute pyelonephritis was established by the method of Freedman and Beeson (10) by injection of *E. coli* into the renal medulla on the side of unilateral ureteral ligation. The acute pyelonephritis involved the renal parenchyma dif-

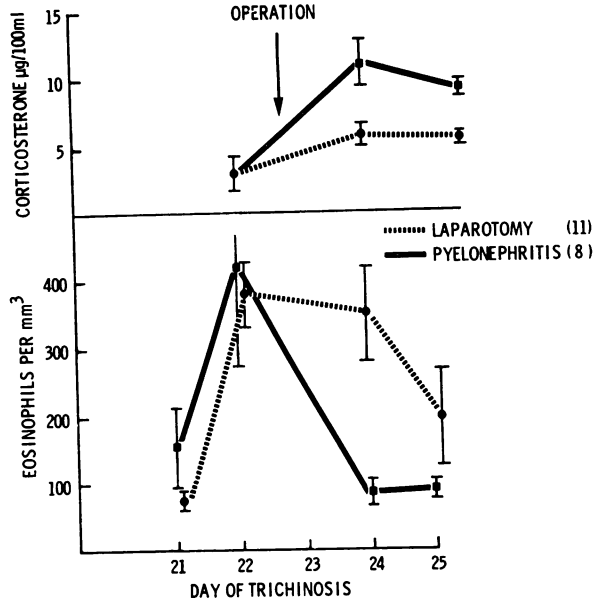

FIGURE 3 Production of pyelonephritis in mice with trichinosis. Serum corticosterone and eosinophil counts after ureteral ligation and intrarenal *E. coli* inoculation or laparotomy without infection (mean±SEM).

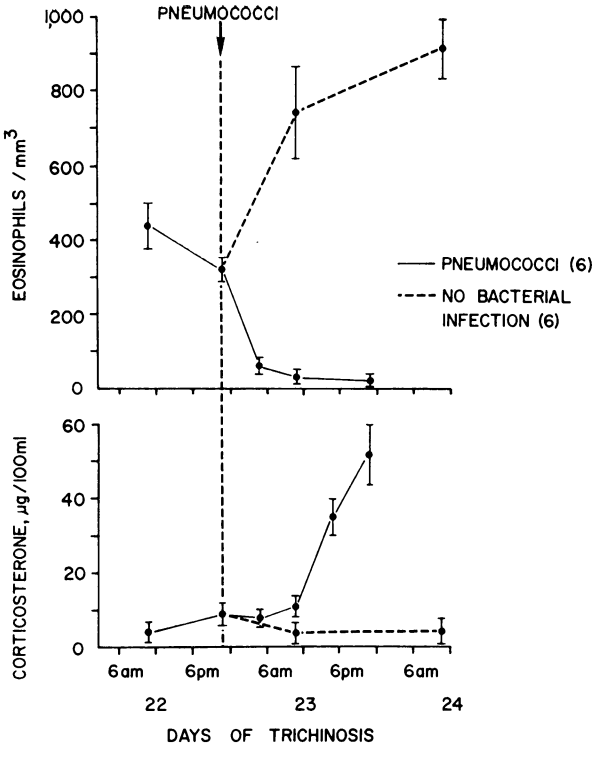

FIGURE 4 Response to pneumococcal abscess in mice with trichinosis. Serum corticosterone and eosinophil counts after inoculation of subcutaneous air pouches with pneumococci or saline (mean±SEM).

fusely and was of reproducible severity. Pyelonephritis was induced on day 21 of trichinosis, and the eosinophil counts were determined 48 and 72 h later. This interval allowed recovery of the mice from the stress of laparotomy. By 48 h postoperatively, eosinophil counts in control animals with trichinosis had returned to elevated levels, whereas those of the infected animals had dropped into the normal range (Fig. 3). Suppression of eosinophilia by acute pyelonephritis persisted for the duration of the experiment. Serum corticosterone concentrations in the mice with acute pyelonephritis rose to a peak of 11.1±1.6 µg/100 ml (mean ± SEM) at 48 h postinfection (Fig. 3). This peak concentration of corticosterone is less than the 13.5±1.5 µg/100 ml peak observed on the 13th day of trichinosis at the time of the first peak of eosinophilia.

Responses of eosinophils to pneumococcal infection. The pneumococcal air pouch abscess is an infection of abrupt onset, and inoculation of pneumococci into a previously established air pouch can be accomplished rapidly without appreciable stress. A reproducible infection results and provides a period of 12–16 h with minimal stress before the onset of clinically apparent illness. Mice were infected with pneumococci on the 22nd day of trichinosis. As rapid sampling was required, separate groups of mice were used to obtain specimens at each time required. After inoculation of the pneumococci at 11:00 p.m., the peripheral eosinophil count fell from 322±23/mm³ to 65±9/mm³ over a period of 6 h. In the control animals, eosinophil counts during this period rose steadily due to normal diurnal variation and to the continuing stimulus of trichinosis (Fig. 4). Although an eosinopenic response occurred during the first few hours of pneumococcal infection, the serum corticosterone concentration did not increase during this period. After 12 h, corticosterone concentration rose abruptly and reached a peak of 54.5±5.8 µg/100 ml after 24 h, when the animals were beginning to succumb.

The eosinopenia of acute inflammation in adrenalectomized mice. Mice were adrenalectomized on the 21st day of trichinosis. Peripheral eosinophil counts were determined on day 22, and the mice were separated into equivalent groups. On the evening of day 22, pneumococci were inoculated into previously established air pouches, and eosinophil counts were obtained 12 h after inoculation. All of the infected mice developed eosinopenia (Fig. 5). The completeness of adrenalectomy was verified by determination of corticosterone concentrations in all terminal blood samples. At the time of final sampling, most of the mice were mildly ill, had ruffled fur, and were somewhat less active than normal mice.

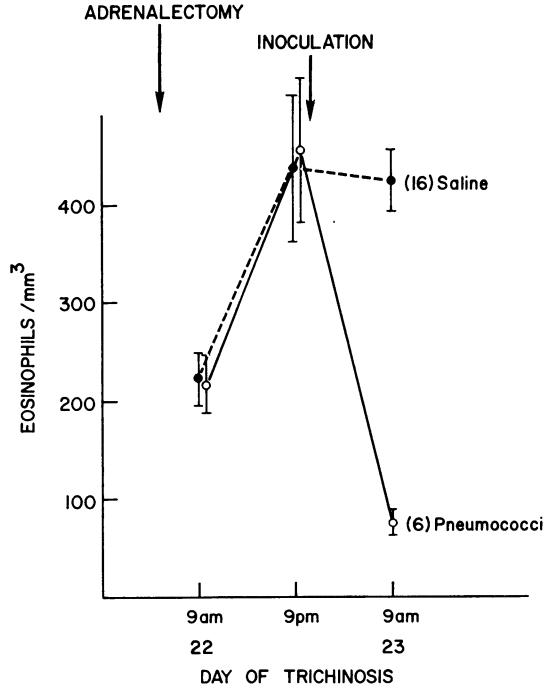

FIGURE 5 Eosinophil counts in adrenalectomized mice given a subcutaneous air pouch inoculation of pneumococci or saline (mean±SEM).

TABLE I

Effect of Pneumococcal Infection on Eosinophil Counts in Peripheral Blood of Adrenalectomized and Control Mice with Trichinosis

Pretreatment group	Eosinophils/mm³					
	Uninfected mice			Infected mice		
	No. of mice	Mean	SEM	No. of mice	Mean	SEM
Controls without adrenalectomy	13	681	112	5	75	19
Interval between adrenalectomy and acute infection:						
1 day	15	217	28	5	73	15
2 days	16	435	42	6	53	11
3 days	10	279	72	11	181	46
4 days	6	362	69	6	84	16

All eosinophil counts were measured on the 23rd or 24th day of trichinosis at 12 h after injection of subcutaneous air pouches with pneumococci or with sterile saline.

The few mice that were severely ill were excluded from the experiment.

To ensure that the eosinopenic response was not dependent on small amounts of corticosteroids remaining after adrenalectomy, the interval between adrenalectomy and subsequent pneumococcal infection was varied from 1 to 4 days. In each case, mice were infected with pneumococci on the evening of day 22 of trichinosis, and eosinophil counts were determined 12 h later (Table I). Characteristic eosinopenic responses were demonstrated in all groups, although the eosinophil counts in the mice studied 3 days after adrenalectomy were quite variable. The differences in eosinophil counts in infected and control mice were statistically significant ($P < 0.05$) in all groups. Control mice with active trichinosis began to appear ill when kept for more than 4 days postadrenalectomy and were considered unsuitable for further study.

DISCUSSION

The objectives of this investigation were to develop reproducible experimental systems for studying the responses of eosinophils to acute infections and to evaluate the widely accepted but unproven hypothesis that the eosinopenia associated with acute infections is the consequence of adrenal stimulation. The hypothesis of adrenal mediation requires that the adrenal response to diseases associated with eosinophilia is minimal and that marked adrenal stimulation occurs in conditions associated with eosinopenia. This study examined such adrenal responses in mice. As with man, the mouse develops eosinopenia after exposure to stress or after injection of exogenous adrenocorticotrophic hormone, and this eosinopenic response is blocked by prior bilateral adrenalectomy (19). Corticosterone is the physiological glucocorticoid hormone in mice, and exogenous corticosterone produces a maximal eosinopenic response (20). The development of a sensitive assay for corticosterone in serum facilitated this study in mice. Experimental trichinosis provided a useful model for examining the corticosterone response in a condition associated with eosinophilia. Serum corticosterone concentrations in mice with trichinosis rose to approximately twice normal levels at the onset of clinical symptoms, then gradually subsided to normal over the next 10 days, even with persistence of the intense inflammatory response to encysting larvae. Similar changes in corticosteroid concentrations in serum have been observed in experimental animals and in man during mild bacterial, viral, and rickettsial infections (21–24). Plasma corticosteroids rise strikingly only when a severely stressful complication such as septic shock is present (24, 25). These observations support the suggestion (23) that many different infections elicit a stereotyped adrenal response, and our data show that trichinosis may now be added to this group of diseases.

The plasma corticosterone response during *Escherichia coli* pyelonephritis was not greater than that induced during the first peak of eosinophilia in trichinosis, but pyelonephritis induced eosinopenia instead of eosinophilia. Pneumococcal infection of a subcutaneous air pouch provided a useful experimental model for observing the eosinophil responses at early times after infection. The circulating eosinophil counts dropped markedly within 6 h after inoculation and continued to fall at 12 h postinfection. Yet there was no significant increase in serum corticosterone concentrations at 6 or 12 h after pneumococcal infection. This suggests that the eosinopenia developed before the onset of adrenal stimulation. Although it may be argued that the serum concentration of a hormone may not accurately reflect the rate of hormone production, it seems doubtful

that there would be no rise in serum corticosterone in the presence of significant adrenal stimulation. To provide more definitive data, the studies were repeated in adrenalectomized mice. The characteristic eosinopenic response to early pneumococcal infection also occurred in mice devoid of adrenal tissue. The possibility that any residual adrenal steroids exerted a permissive effect was minimized by studies of animals infected up to 4 days after adrenalectomy.

The eosinopenia of acute infection is not mediated by adrenal stimulation and subsequent suppression of circulating eosinophils by corticosteroids. The eosinopenic response to acute infection is a distinctive aspect of eosinophil physiology.

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