Behavior of Eosinophil Leukocytes in Acute Inflammation

II. EOSINOPHIL DYNAMICS DURING ACUTE INFLAMMATION

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ABSTRACT The marked diminution in the number of circulating eosinophils, which has been shown to occur during acute bacterial infections, is a distinctive aspect of eosinophil physiology and of the host response to acute infection. The mouse rendered eosinophilic by infection with trichinosis provides a suitable model for study of the eosinopenic response induced by acute inflammation. The alterations in eosinophil dynamics associated with acute inflammatory reactions in trichinous mice were studied with pneumococcal abscesses, with *Escherichia coli* pyelonephritis, with Coxsackie viral pancreatitis, and with acute subcutaneous inflammation due to turpentine. Each of these stimuli of acute inflammation markedly suppressed the eosinophilia of trichinosis. This suggests that the eosinopenia is a response to the acute inflammatory process rather than the response to a specific type of pathogen.

These studies apply quantitative techniques to ascertain the effects of acute inflammation on eosinophil production in bone marrow and on distribution of eosinophils in the peripheral tissues. From these observations, it is apparent that the initial response to acute inflammation includes a rapid drop in numbers of circulating eosinophils, a rapid accumulation of eosinophils at the periphery of the inflammatory site, and an inhibition of egress of eosinophils from the bone marrow. With prolongation of the inflammatory process, inhibition of eosinopoiesis occurs.

INTRODUCTION

One of the most distinctive properties of eosinophil leukocytes is the dramatic fall in the number of circulating eosinophils that occurs during an acute infection. This eosinopenia of acute infection has been assumed to be a secondary expression of adrenal glucocorticosteroid stimulation produced by the stress of the infection. Hence, the phenomenon has not been re-examined since 1934, when Spink described suppression of the eosinophilia of trichinosis in guinea pigs with staphylococcal, tuberculous, or trypanosomal infections (1). Recent studies from this laboratory demonstrated that mice with trichinosis develop a reproducible eosinophilia that provides a suitable base line for the study of eosinopenia induced by acute inflammatory processes (2, 3). The suppression of the eosinophilia of trichinosis during pneumococcal infection was found to occur independently of adrenal stimulation and in adrenalectomized mice (3). These studies indicated that the eosinopenia that occurs during acute infection cannot be ascribed solely to adrenal stimulation and that it represents a distinctive aspect of the host response to acute infection.

Previous studies demonstrated an eosinopenic response during acute bacterial infections (1-3). The present studies show that acute inflammation caused by viral pancreatitis and by subcutaneous injection of turpentine produces a similar decrease in numbers of circulating eosinophils.

Before undertaking studies to define the mechanism and physiologic significance of the eosinopenic response to acute infection, it was desirable to apply currently available quantitative techniques to define further the nature of the phenomenon. The possibilities included the following: (*a*) a peripheral sequestration of eosinophils by localization in such sites as the inflammatory region, the draining lymph nodes, or the spleen, by diffuse intravascular margination, or by destruction of eosinophils; (*b*) a suppression of egress of mature eosinophils from the bone marrow; and (*c*) a suppression of eosinophil production. The current studies examine these possibilities.

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METHODS

Unless stated, the experimental methods are the same as described previously (3).

Animals. Inbred C3H/mg mice were obtained in a pathogen-free condition from the Medical Research Council Laboratory Animal Centre, Carshalton, Surrey, England and Scientific Agribusiness Consultants International Ltd., Braintree, England.

Bone marrow preparation. Quantitative cell counts on femoral bone marrow were performed by a modification of the method of Chervenick et al. (4). The mice were anesthetized with ether, peripheral blood was sampled, and the mice were then killed by cervical dislocation. Both femurs were removed and cleaned of attached muscle. Femoral ends were removed by carefully nibbling with bone forceps until the marrow cavity was just exposed. A no. 23 needle was inserted in the distal end and 2 ml of (Eagle's) minimal essential medium (MEM) [1] (Biocult Labs, Glasgow, Scotland) containing 5 U of heparin/ml was forced through the cavity, expelling most of the marrow as a plug. The fluid was then drawn back and forth through the femur to remove any remaining cells. A free cell suspension was made by drawing the entire sample through a no. 23 needle several times. The cells from both femurs were pooled. The total white cell count and eosinophil count of the 2 ml of fluid were then determined as for whole blood. The eosinophil counts were made within 20 min of diluting marrow suspensions in counting fluid, as some of the immature marrow cells would not lyse. Although such cells were at first easily distinguished by their size and lack of uptake of eosin, they later absorbed enough dye to make identification less certain. For differential counts, one or two drop samples of the marrow preparation were spun in a cytocentrifuge (Shandon Ltd., London) at 500 *g* for 5 min onto microscope slides previously acid-alcohol cleaned. These were stained with hematoxylin and Biebrich scarlet and examined as previously described (5).

Labeling of bone marrow cells with [^3H]thymidine. Incubation with [^{3}H]thymidine in vitro provided an indication of the number of marrow cells synthesizing DNA (a reflection of the "mitotic pool"). Suspensions of femoral marrow cells were prepared as described and 10% fetal bovine serum (Biocult Labs) was added to the medium. This technique of bone marrow preparation provided a suspension of cells with greater than 95% viability as demonstrated by trypan blue exclusion. To each 2-ml marrow suspension, 1 μCi of [^{3}H]thymidine (45 Ci/mmol, The Radiochemical Centre, Amersham, England), in 50 μl of MEM was added, and the samples were then incubated at 37°C in a water bath with gentle horizontal agitation for 1 h exactly. To stop uptake of thymidine, 15 ml of cold MEM was added to each tube, and the samples were washed three times with 15-ml aliquots of MEM with centrifugation at 400 *g* for 10 min between washings. Finally, several cytocentrifuge preparations on microscope slides were made from each sample as described above. These were fixed in methanol and lightly stained with Harris' hematoxylin (staining with hematoxylin before autoradiograph preparation reduced the chance of later loss of emulsion granules during staining). The preparations were dipped in nuclear emulsion K2 (Millford Ltd., Essex, England) which had been melted at 50°C and diluted 1:1.5 with distilled water. Slides were then held at 4°C for 3 wk in light-tight boxes. They were developed in Kodak D19 solution (Kodak Ltd., Hemel Hempstead, Herts., England) and fixed with standard biosulfate hypo. They were then stained with Biebrich scarlet as described below and examined on the same day. Differential counts of labeled and unlabeled cells were made and a minimum of 200 cells of the specific cell type under study was counted (500 cells in the case of the marrows from eosinophilic mice).

Pneumococcal infection. Production of a 2.5-ml subcutaneous air pouch and inoculation with 2×10^8 log-phase type 3 pneumococci were performed as described previously (3). This was satisfactory for acute experiments, but survival of the mice was too short for more chronic study. As observed previously in rats (6), a curtailment of bacterial growth by sulfapyridine resulted in a balance between host defenses and bacterial virulence prolonging the infectious process without killing the mice. A dose of 25 mg sulfapyridine (May and Baker, Dagenham, Essex, England) was given orally under light anesthesia by means of intubation with a blunted no. 11 needle, beginning at 9 h after bacterial inoculation and continuing at intervals of 12 h. Mice thus treated appeared only slightly ill, ran freely about the cage, and ate sufficiently to maintain weight. After 4 days these mice had developed a ring of edematous, gelatinous, subcutaneous material up to 2 mm in thickness that contained 4×10^9 pneumococci/g and frequently extended completely around the abdomen. There was continuous bacteremia from 18 h onward, with 10^4–10^6 organisms/ml of blood. Histologically, all organs (lung, liver, spleen, and kidney) were free of evidence of localized inflammatory processes or abscesses. During treatment with sulfapyridine, this huge bacterial load had remarkably little apparent effect on the mice.

Reproducible cure of the pneumococcal infections was produced by the administration of 4-mg doses of procaine penicillin (Distaquaine G, Dista Products Ltd., Liverpool) subcutaneously at 12-h intervals for four doses.

Staphylococcal infection. Staphylococcus aureus type PS80 was supplied by Dr. Parker (Public Central Health Laboratories, Colindale, London). This organism was stored on an agar slope at 4°C. When a culture was required, it was first grown overnight on blood agar and one colony was inoculated into 15 ml brain-heart infusion broth and incubated at 37°C for 6 h with agitation.

Reproducible abscesses were produced by subcutaneous implantation of small plugs of compressed cotton dust impregnated with staphylococci by the method of Noble (7). With an inoculum of 10^6 organisms, the mice developed small abscesses 3–6 mm in diameter which progressed over 2–5 days to form a small necrotic slough of skin, abscess drainage, and eventual healing. There were no instances of disseminated staphylococcal infection.

Coxsackie viral pancreatitis. Coxsackie B4 virus was supplied by Dr. D. R. Gamble (Coxsackie Reference Laboratory, Public Health Laboratories, Westpark Hospital, Epsom, Surrey, England) in a mouse torso suspension of the eighth passage of a suspension of pancreas in suckling mice. It had a titer of $10^{-6.5}$ LD_{50} in newborn mice, and produced massive necrosis of pancreatic acinar tissue. For infections in 7-wk-old C3H/mg mice, an intraperitoneal infective dose of 50 μl of a 10^{-1} dilution was selected and produced an active pancreatitis in all animals inoculated, with an eventual mortality of 30%. After an incubation period of 8 days, the mice appeared mildly ill (ruffling of fur). Mice sacrificed at this stage had multiple scattered areas of acute pancreatic inflammation without histologically apparent involvement of the liver or myocardium. Deaths

[1] *Abbreviation used in this paper:* MEM, minimal essential medium.

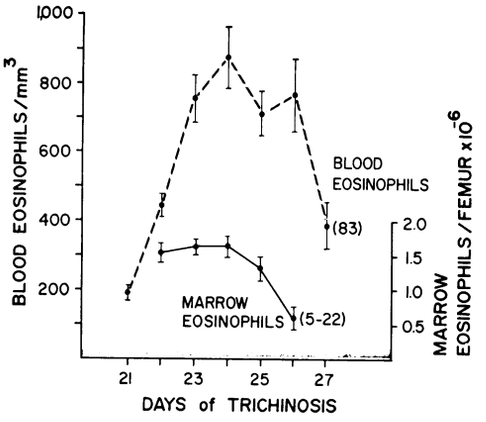


FIGURE 1 Eosinophil counts in the peripheral blood and total eosinophils in the femoral bone marrow in mice between 21 and 27 days after oral inoculation with *Trichinella spiralis* larvae (mean±SEM). The shaded area represents the normal range for total marrow eosinophils in C3H/mg mice.

occurred between the 8th and 13th days. By the 15th day, inflammation had subsided but several mice had developed progressive wasting and had no identifiable pancreatic tissue when autopsied. When the same inoculum was given to mice on the 15th day of oral trichinosis, they followed a more severe course. After 8 days the mice became abruptly ill and 9 of 12 succumbed by the 13th day. Autopsies demonstrated only acute pancreatic inflammation without involvement of the liver or myocardium; areas of acute inflammation also existed in some parts of the perirenal fat and omental fat, presumably due to release of pancreatic enzymes.

Splenectomy. After anesthetizing with Nembutal, the abdomen was opened anteriorly. A cotton wool swab was inserted and twisted gently to secure the omentum. As this was pulled out, the spleen followed. Splenic hilar vessels and splenogastric ligament were ligated and severed. Peritoneum was closed with a 4/0 silk suture and the skin repaired with 9-mm steel clips (Autoclips, Clay-Adams, Inc., Parsippany, N. J.).

Histological methods. Tissues for histological study were fixed in 10% buffered formal saline for 24 h, washed several times in 70% alcohol, and processed on an automatic Histokinette machine for embedding. They were sectioned at 5 μm thickness, dried overnight at 45°C, dewaxed in xylene, passed through 100, 95, and 70% alcohol and into water. They were then strained with standard Ehrlich's hematoxylin. The counterstain was the following modification of the standard Biebrich scarlet method. The use of Biebrich scarlet has proven very valuable for identification of eosinophils in tissues in most species (e.g., see reference 5, for use in rat tissues). Mouse eosinophils stain less well than those of other species and in the standard method the stain is likely to be lost during the decolorization process. The sections were stained for a minimum of 30 min in a saturated solution of water-soluble Biebrich scarlet in Sørenson glycine-NaOH buffer at pH 9.2. They were very briefly washed in water, passed quickly through 70, 95, and 100% alcohol with a 1-min maximum in each solution, and finally into xylene before mounting. This results intentionally in somewhat inadequate decolorization with a mild pink color remaining in the cytoplasm of most cells, a red color to any red cells present, and a brilliant crimson in the granules of eosinophils. For staining of films of cells such as marrow preparations, the procedure was the same except that passage through alcohol was limited to a 10-s dip in 70% alcohol, quickly followed by washing with water and drying.

Basic experimental design. As discussed previously (3), a study of stimuli which induce eosinophil leukopenia requires the availability of a predictable and reproducible eosinophilia as a base line to facilitate statistcal analysis of the changes in the leukocyte population. The second peak of eosinophilia induced by trichinosis in mice was used in most of the experiments in the study. Migration of trichinella larvae from the intestinal tract occurs before and during the first peak of eosinophilia (8). Acute inflammatory stimuli were superimposed after larval migration to striated muscles, thus minimizing any possible effect on the trichinella infection itself. The usual procedure was to determine the animals' response on day 22 of trichinosis, at which time the eosinophil level is beginning to rise (Fig. 1). Having verified that the eosinophilic response was underway, the animals were randomized and the inflammatory stimuli were applied. Experimental and control groups were compared on days 23 and 24 of trichinosis at the expected height of eosinophilia. This timing was also advantageous for studying changes within the bone marrow, as the marrow eosinophils remain at a constant level during this period (Fig. 1).

RESULTS

Normal values. Normal hematological values in C3H/mg mice are shown in Table I. As has been noted previously, these are characterized by a low resting state of eosinophil production, as shown in both peripheral blood and bone marrow.

Responses of peripheral blood eosinophils to inflammatory stimuli. Coxsackie B4 pancreatitis provided a model of viral infection characterized by predominantly acute inflammatory response. The inoculum of Coxsackie B4 virus that was chosen produced pancreatitis in mice after a reproducible incubation period of 7–8 days. The mice were therefore inoculated on day 15 of trichinosis, so that the anticipated peak eosinophil response would coincide with the onset of pancreatitis. Although the majority of infected mice displayed minimal signs of systemic illness on days 7 and 8 of the infection, the eosinophilia of trichinosis was completely suppressed at that time (Fig. 2).

Turpentine provided a noninfectious acute inflammatory stimulus. The effect of injecting 0.1 ml of turpentine subcutaneously on day 22 of trichinosis is shown in Fig. 3. As with acute infectious processes, a dramatic fall in circulating eosinophils occurred promptly and persisted for several days.

Studies on localization of eosinophils during eosinopenia induced by acute inflammation. The studies of changes in peripheral blood have shown a remarkably rapid fall in the number of circulating eosinophils after induction of an acute inflammatory process. This was

TABLE I

Hematological Values in Normal C3H/mg Mice

	Number of mice	Mean	SD	SEM
Peripheral blood				
Eosinophils/mm³	90	65.2	29.8	3.5
Neutrophils/mm³	21	2,139	1,085	236
Lymphocytes/mm³	21	4,958	2,237	488
Bone marrow: total cells				
Eosinophils/femur $\times 10^{-5}$	14	0.774	0.254	0.068
Neutrophils/femur $\times 10^{-5}$	11	90.9	29.5	8.9
Bone marrow: [³H]thymidine labeled				
Eosinophils/femur $\times 10^{-5}$	8	0.206	0.066	0.023
Neutrophils/femur $\times 10^{-5}$	12	15.5	6.35	1.83

especially apparent in the early response to pneumococcal abscess (3). The rapidity of the fall in circulating eosinophils suggests that a sudden peripheral sequestration may be an early manifestation of acute inflammation. The site of this postulated sequestration has not been determined, but the most probable sites would be the spleen (9-12), the inflammatory lesion itself (13) or its draining lymph nodes (14), or diffuse intravascular margination.

To investigate the role of the spleen, mice were splenectomized on day 21 of trichinosis. On day 22, their peripheral eosinophil counts were found to be 1,480± 236 eosinophils/mm³ (mean ±SEM, $n = 12$ mice), more than twice that of their nonsplenectomized littermates. Pneumococcal abscesses were established at 11:00 p.m. on day 22 of trichinosis and eosinophils were counted 10 h later (Fig. 4). The resultant eosinopenia in the splenectomized mice, a drop from 1,497 to 24±9/mm³, was even greater than that seen for normal animals studied concomitantly, i.e., from 540 to 90±15/mm³. Rather than aborting the mechanism of eosinopenia, the removal of the spleen and its store of mature eosinophils had enhanced it.

The possibility that eosinophils might be drawn into the lymph nodes draining the site of the acute inflam-

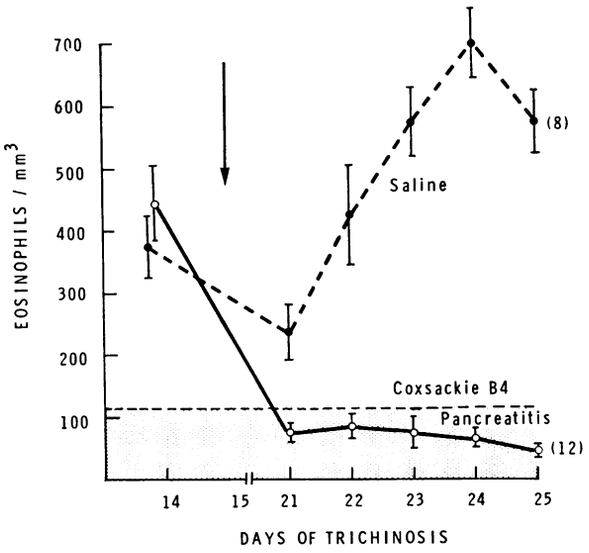


FIGURE 2 Eosinophil counts of mice given intraperitoneal Coxsackie B4 on the 15th day of trichinosis (↓) (mean ±SEM).

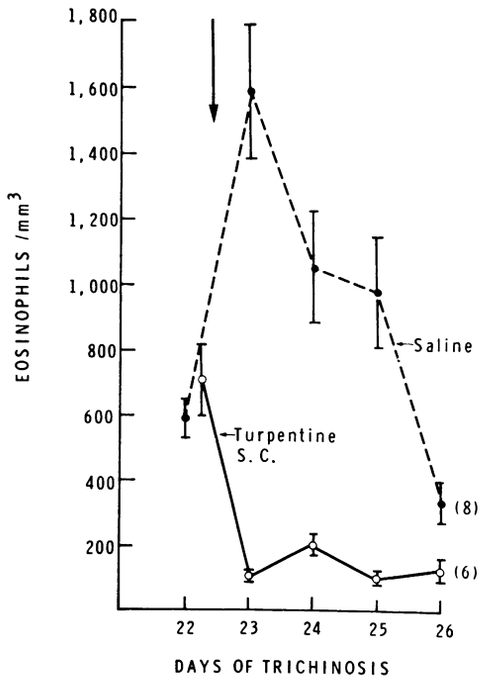


FIGURE 3 Eosinophil counts in the peripheral blood of mice injected subcutaneously with 0.1 ml of turpentine on day 22 of trichinosis (↓) (mean±SEM).

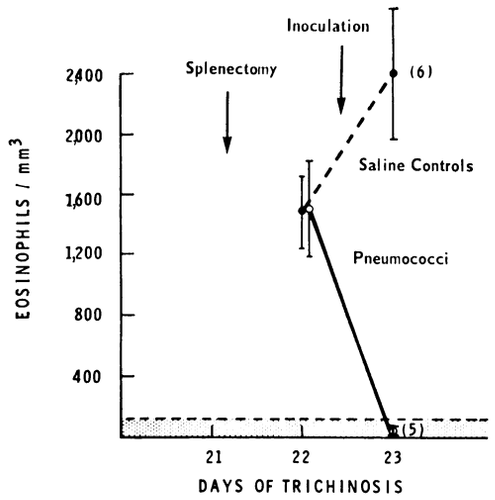


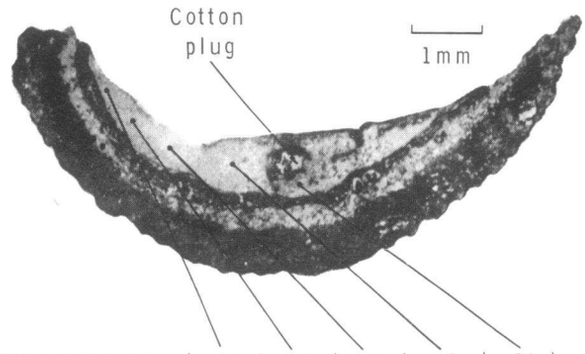
FIGURE 4 Eosinophil counts in the peripheral blood of mice given splenectomy on day 21 (↓) and subcutaneous air-pouch injection with pneumococci or saline on day 22 of trichinosis (↓) (mean±SEM).

matory process was examined histologically. Mice on day 23 of trichinosis, with a peripheral eosinophilia of over 1,000/mm³, were given pneumococcal abscesses over the dorsal thoracic region. 8 h later, the axillary nodes were dissected and processed for staining with hematoxylin and Biebrich scarlet. There were relatively few eosinophils demonstrable in the nodes of the control mice and no appreciable accumulation of eosinophils in the nodes from the mice with the pneumococcal abscess.

Eosinophils appeared to congregate around the periphery of the acute inflammatory response in histological sections of mice with pneumococcal abscesses or Coxsackie viral pancreatitis. However, the widespread nature of the pneumococcal infection and the extensive but irregular involvement of the pancreas in Coxsackie pancreatitis made it difficult to quantify this phenomenon. In contrast, subcutaneous abscess formed by a small plug of cotton dust impregnated with staphylococci provided a localized acute inflammatory lesion that could be excised en bloc and subjected to more precise histopathological examination. Subcutaneous cotton-plug abscesses were produced in mice on day 23 of trichinosis with peripheral eosinophil counts over 1,000/mm³. 7 h later, the regions containing the cotton plugs were excised and processed for histological study. Sections through the abscess center were prepared with a thickness of 5 μm and stained with hematoxylin and Biebrich scarlet. It was observed that numerous eosinophils had already accumulated at the region of the abscess site. To estimate the distribution of polymorphonuclear leukocytes in several regions of the inflammatory lesion, neutrophils and eosinophils were enumerated with the help of an eyepiece reticle that had been calibrated with a stage micrometer and covered an area of 0.11 mm². The numbers of cells found within such areas at varying distances from the abscess center are shown in Fig. 5. The data are shown for only one tissue section, but a similar distribution was found in each of the eight abscesses examined. Immediately adjacent to the cotton plug, neutrophils and eosinophils were numerous and were present in a ratio similar to that observed in the peripheral blood before establishing the abscesses. The absolute numbers of neutrophils rapidly diminished as the distance from the abscess center increased. In contrast, the number of eosinophils increased at the periphery of the inflammatory process. In each of the eight abscesses examined, the number of eosinophils was greater at the periphery (2.5–3.5 mm from the abscess) than near the abscess center.

An estimate of the total number of eosinophils at the abscess site was derived from the above data by assuming that the subcutaneous abscess had the shape of a half sphere with a concentration of eosinophils varying with distance from the abscess center as shown in Fig. 5. Such an estimate would suggest that 7×10^6 eosinophils had accumulated at the abscess site 7 h after inoculation. The eosinophil count of the same mouse at the time of inoculation was 1,600/mm³, indicating a total circulating pool of approximately 2.2×10^6 eosinophils. It appears that the number of eosinophils found at the abscess site may be sufficient to account for the initial drop in numbers of circulating eosinophils after induction of an acute infection.

Responses of marrow eosinophils to inflammatory stimuli. As the characteristic alterations in peripheral



EOSINOPHILS	77	63	20	18	46
NEUTROPHILS	11	10	47	132	288
% Eos	88	86	30	12	14

FIGURE 5 Distribution of eosinophils and neutrophils near an abscess of 7 h duration. The cotton plug, impregnated with staphylococci, was inserted subcutaneously in a mouse with eosinophilia. Cell counts are for areas of 0.11 mm². Section thickness, 5 μm.

blood during acute inflammation involve a reduction in the circulating eosinophils of sudden onset but prolonged duration, possible changes in marrow might include cessation of egress of mature eosinophils into the blood or cessation of eosinophil production or both, or no change in production or egress of marrow eosinophils, reflecting a peripheral phenomenon. The initial studies of eosinophil kinetics were performed with mice stimulated by trichinosis to a stage of predictable eosinophilia so that statistically significant changes in eosinophil counts could be more easily documented. Quantitative techniques for marrow analysis were used in all experiments.

The effect of pneumococcal abscesses on total marrow eosinophil populations as a function of time after pneumococcal infection was determined using a total of 75 experimental and control mice with trichinosis (Fig. 6). The duration of the acute pneumococcal infection was varied by adjusting the time of bacterial inoculation so that marrow sampling in all mice could be done at the same time of day on either day 23 or 24 of trichinosis, thereby assuring constant marrow eosinophil counts in the controls. The eosinophil counts of the stimulated marrow in these trichinous control animals were 30 times the mean of counts from unstimulated mouse marrows. Nevertheless, during the first 36 h of acute pneumococcal infection, a further doubling of the total marrow eosinophils was observed. This was followed by a decline in marrow eosinophil counts toward normal levels during the next 72 h.

The early increase in marrow eosinophils after pneumococcal infection could be due either to sudden cessation of egress of mature eosinophils from the marrow or to a burst of eosinophil proliferation. If the phenomenon were due to an inhibition of marrow egress with resultant increase in the number of mature eosinophils, the number of eosinophils synthesizing DNA should be unaffected. In contrast, a stimulation of eosinopoiesis

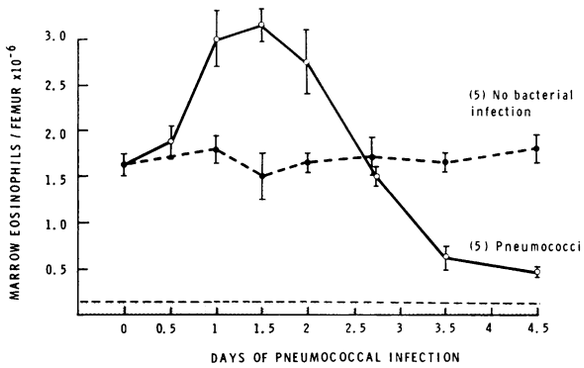


FIGURE 6 Total femoral marrow eosinophils in mice with a subcutaneous pneumococcal infection prolonged during sulfapyridine treatment (mean±SEM). All marrows were examined on the 23rd or 24th day of trichinosis.

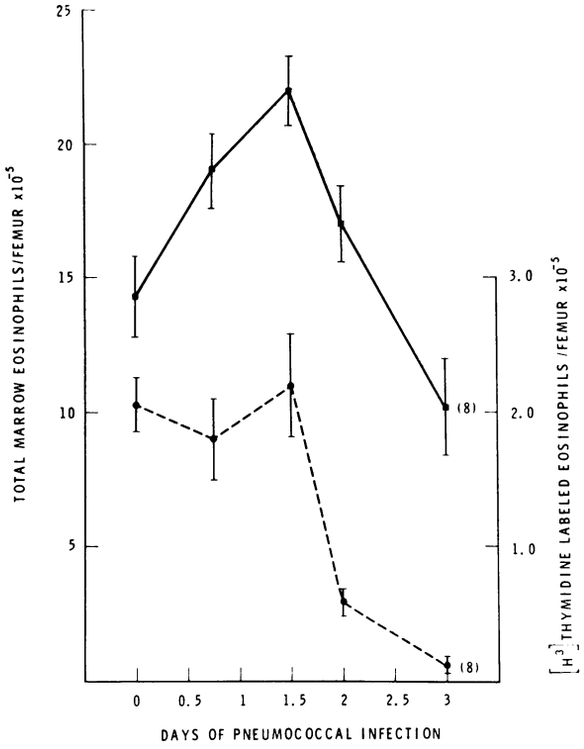


FIGURE 7 Total marrow eosinophils (■) and marrow eosinophils labeled in vitro with [³H]thymidine (●) in mice with pneumococcal infection prolonged by treatment with sulfapyridine (mean±SEM). All marrows were examined on the 23rd or 24th day of trichinosis.

should be reflected by an increasing number of eosinophils synthesizing DNA, which could be estimated by pulse-labeling with [³H]thymidine (5). The preceding experiment with pneumococcal abscess was therefore repeated, but in addition, uptake of [³H]thymidine by marrow eosinophils after incubation for 1 h in vitro with [³H]thymidine and autoradiograph preparation was measured. The changes in total marrow eosinophils were the same as previously observed. In contrast, the number of eosinophils labeled with [³H]thymidine did not increase, but remained constant for the first 36 h and then fell rapidly toward normal levels (Fig. 7). Thus, the initial rise in marrow eosinophils was not accompanied by increased eosinophil production, but appeared to be due to an increase in the number of nondividing eosinophils, presumably because of reduced egress of mature cells into the blood. Inhibition of marrow eosinophil production was a late event, and became prominent only after the first 36 h of acute infection.

The animal with trichinosis is in an unusual state of stimulation of eosinophil production. The induction of an acute inflammation inhibits this process and both marrow eosinophil production and total marrow eosinophil counts declined toward normal. To study the effect

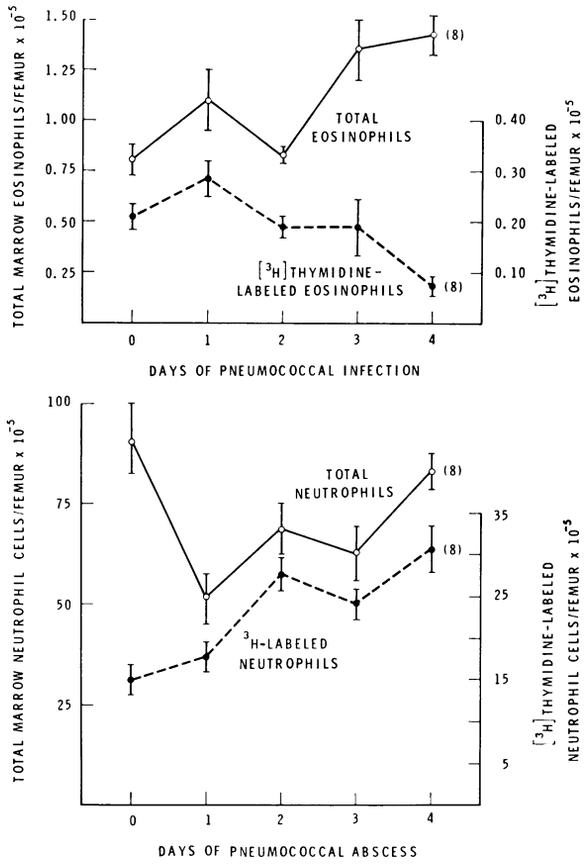


FIGURE 8 Total and [³H]thymidine-labeled eosinophils and neutrophils in normal mice with pneumococcal infection prolonged by treatment with sulfapyridine (mean±SEM).

of acute pneumococcal infection on unstimulated marrow, experiments were performed exactly as before, except that nontrichinous mice free of previous inflammatory or hypersensitivity processes were used. Both the total marrow eosinophil counts and eosinophil mitotic pool were studied simultaneously (Fig. 8). A transient rise in total marrow eosinophils was observed during the 24 h after infection. This was accompanied by a brief but significant increase in the population of eosinophils synthesizing DNA ($P < 0.05$), and this early increase in dividing eosinophils was comparable to the simultaneous early increase in neutrophil production (Fig. 8). After this brief stimulation, however, the eosinophil production declined to less than 40% of "resting" control levels, although the total marrow eosinophil counts gradually increased. This accumulation of nondividing eosinophils may reflect greater inhibition of egress than of production of marrow eosinophils.

The preceding experiments on marrow eosinophil changes were all performed with the test system of subcutaneous pneumococcal abscess. The models of acute *E. coli* pyelonephritis and subcutaneous injection of turpentine provide an opportunity to test whether or not the events observed in the marrow occur after other inflammatory stimuli. Mice were studied on day 23 or 24 of trichinosis and the interval between inoculation of the acute inflammatory agent and sampling of the bone marrow was varied as in the previous experiments. Pyelonephritis was induced by unilateral ureteral ligation and immediate intrarenal inoculation of 10^5 *E. coli* as described previously (3). The initial rise in marrow eosinophils varied in magnitude with the different inflammatory stimuli (Fig. 9). The height of this transient rise was most marked with pneumococcal infection, moderate with *E. coli* pyelonephritis, and negligible with turpentine injection. Nevertheless, all were sufficiently powerful stimuli of acute inflammation to bring about marked depression of eosinopoiesis within 3 days.

Resumption of eosinophilia after cessation of acute inflammation. To determine whether or not the suppression of eosinophilia by inflammation is reversible, the effects of penicillin therapy on eosinophil responses to pneumococcal infection were studied. Pneumococcal air-pouch abscesses were established in mice on day 22 of trichinosis. Six groups of mice were used so that no animal would have its blood sampled more frequently than at 18-h intervals. The first of four injections of penicillin was administered 12 h after infection (Fig. 10). The eosinophil levels continued to fall during the first 6 h of therapy, but then rose over the next 6 h toward the level of the uninfected controls. Thus, the suppressive effects of acute inflammation on the eosinophilia of trichinosis is rapidly reversible when the inflammatory stimulus is removed.

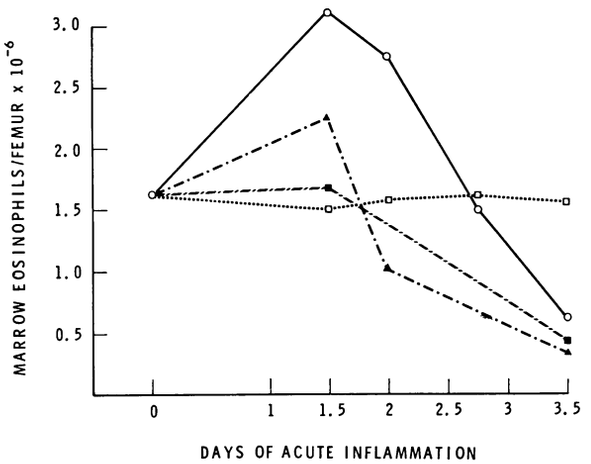


FIGURE 9 Total marrow eosinophils in mice with trichinosis subjected to prolonged pneumococcal infection (○), *E. coli* pyelonephritis (▲), subcutaneous turpentine (■), or subcutaneous saline (□). Marrows were all examined on the 23rd or 24th day of trichinosis. Each point is the mean of five to eight mice.

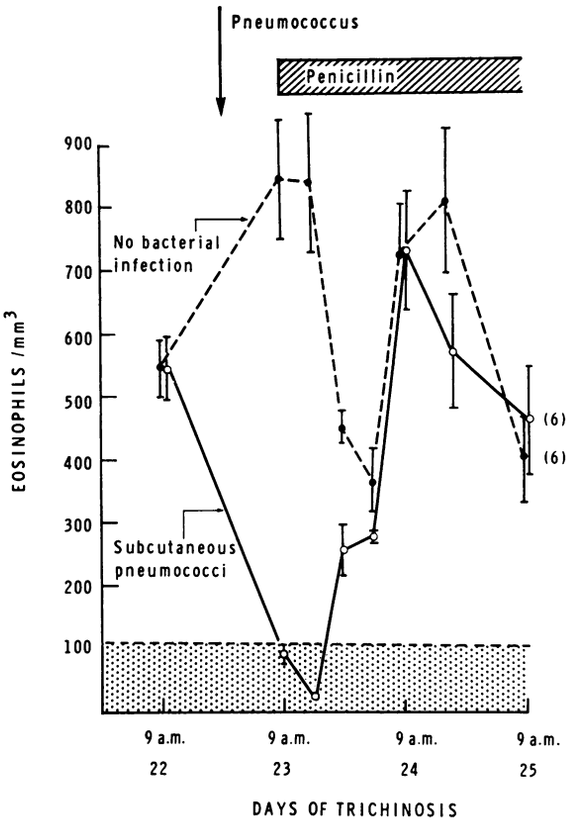


FIGURE 10 Resumption of eosinophilia with cure of the pneumococcal infection (mean±SEM). Mice on day 22 of trichinosis given pneumococcal abscess. Penicillin administered 12 h later. Eosinophil changes in controls due to diurnal variation.

DISCUSSION

These experiments have again confirmed that an acute inflammatory process may obliterate a high eosinophilia with a rapid return of eosinophil counts into the normal range. The eosinopenic responses to pneumococcal abscess and to *E. coli* pyelonephritis (3) and to Coxsackie viral pancreatitis demonstrate that the effect is not due to infection with a specific pathogen or to the elaboration of any known toxin. The effect of turpentine suggests that it is not dependent on infection by a living organism. The one common element in the several models examined appears to be the host response, the acute inflammatory reaction and the systemic reactions induced by it.

The eosinopenic response to acute inflammation is remarkably rapid. The induction of a subcutaneous pneumococcal abscess was observed to produce an 80% fall in the peripheral eosinophil counts within 6 h (3). After splenectomy, the eosinopenic response was even more dramatic, the peripheral eosinophil counts falling more than 98% in 10 h. Although the blood transit time of eosinophils is not known for the mouse, studies in the rat have demonstrated it to be between 6.6 and 19.2 h (12, 15). Unless the blood transit half-life of the eosinophil in the mouse is remarkably rapid, the possibility that the rapid onset of peripheral eosinopenia is due solely to a blockade of bone marrow egress of eosinophils appears untenable. Alternatives would be peripheral intravascular margination of eosinophils or a rapid mobilization of these cells into peripheral tissues during the response to acute inflammation.

The purpose of the study of eosinophil changes in peripheral tissues was to observe such possible sites of early localization of eosinophils. The spleen is thought to act as a storage site for eosinophils (9–11) and can contract after epinephrine administration to release eosinophils into the circulation. Spry (12), while studying rat eosinophil kinetics, found that eosinophils appeared to lodge in the spleen for a final period of maturation before progressing on to the tissues. The rapid eosinopenic response occurring in animals with splenectomy suggests that the spleen, rather than providing a site of sequestration during acute inflammation, probably releases eosinophils during the process and helps to dampen the effect of acute inflammation. Eosinophils are known to enter an area of inflammation in a manner similar to that of neutrophils (13). The large numbers of eosinophils observed in the vicinity of the acute staphylococcal abscess may be sufficient to explain the rapid decline in the numbers of these cells in the peripheral circulation.

The observation that eosinophils collect at the periphery of the inflammatory process is compatible with previous histological studies of acute (16, 17) and granulomatous (18, 19) inflammation. Steele and Wilhelm (17) in a study of chemically induced inflammation observed that, "compared with the neutrophils, eosinophils have a tendency to accumulate rather deeper in the dermis (than the neutrophils) and further from obviously damaged tissue. They are also more numerous in the lateral borders of the lesion as though they were spectators of the struggles of the neutrophils rather than participants." Both eosinophils and neutrophils emigrate into the vicinity of an inflammatory stimulus by diapedesis (13). The peripheral concentration of eosinophils and central concentration of neutrophils in abscesses might be explained in part by a more sluggish response of eosinophils to neutrophil chemotactic agents (20). This hypothesis suggests that neutrophils move rapidly to the inflammatory center, perform their function and become less motile (21, 22) or die, therefore accumulating at the abscess center. In contrast, eosinophils move more slowly toward the chemotactic stimuli at the abscess. Moreover, those found there do not display partial degranulation or histological signs of dying, and may be

able to continue their random motility without accumulating in large numbers at the abscess center. If this hypothesis were true, a relatively even dispersion of eosinophils throughout the inflammatory region would be expected. In fact, however, the greatest concentration of eosinophils occurred 2.5–3.5 mm from abscesses of only a 0.4-mm radius and was peripheral to the capillaries nearest the abscess. This quantitative increase in eosinophils distal to the site of initial emigration of inflammatory cells suggests that the eosinophils are specifically attracted to the periphery of the inflammatory process. Although several substances have been described that are chemotactic for eosinophils, none has been shown to be responsible for such a localization of eosinophils away from the center of the inflammatory stimulus.

Eosinophils have recently been found to produce a substance which inhibits histamine release by basophils (23) and also to produce an arylsulphatase that inactivates slow-reacting substance of anaphylaxis (24). The neutralization of these well-characterized mediators of inflammation as well as the localization of eosinophils in the periphery of the inflammatory process strongly support a teleological speculation proposed in 1914 (18): the eosinophil serves a homeostatic function by inactivating byproducts of inflammation or by reducing sensitivity to further inflammatory stimuli, thus helping to contain the inflammatory process.

The initial bone marrow-eosinophil response to acute inflammation involved an increase in total numbers of marrow eosinophils without an apparent concomitant increase in eosinophil production. This suggests the occurrence of an inhibition of egress of eosinophils from the bone marrow. The rapidity and magnitude of this initial increase in total marrow eosinophils appears to depend on the intensity of the acute inflammatory process and the state of stimulation to eosinopoiesis of the marrow. It did not become definite in the resting marrow until the 3rd and 4th days of the acute inflammation. In the mouse with trichinosis, the effect is dramatic during pneumococcal infection, is appreciable during *E. coli* pyelonephritis, and is minimal during turpentine-induced subcutaneous inflammation. The mechanism of this apparent inhibition of marrow egress is unknown. A possibility to be considered is that a massive release of neutrophils from the marrow (see Fig. 7) prevents the concomitant escape of eosinophils, but such a selective competition between these two cell types for the innumerable possible points of egress from the marrow lacunae into the blood during the early response to acute inflammation seems unlikely. More probable is the interruption of an active process, the normal stimulation for eosinophil release, whatever that may be.

In the presence of acute inflammation, eosinophil production eventually declines in both the resting and stimulated bone marrow. This decline could be due to any of three processes: (*a*) inhibition of a pre-existing stimulus to eosinophil production, (*b*) a direct effect on dividing eosinophils, (*c*) an effect on the unrecognizable eosinophil precursors, the "stem cells," which might be diverted from eosinophil to neutrophil production. The data available cannot distinguish between these possible mechanisms.

The previous observations of Morgan and Beeson (2) demonstrated a similar eventual decrease in total marrow eosinophils as a response to inflammation; however, the early accumulation of marrow eosinophils was not observed during that study. This apparent discrepancy may be due to differences in the experimental production of pyelonephritis and the timing of marrow sampling. The earlier study delayed the inoculation of *E. coli* until 24 h after ureteral ligation and considered this point "time zero." The first marrow samples were taken 48 h later, which would already be somewhat into the depressive phase of the pyelonephritis curve. Furthermore, the preceding surgery may have caused sufficient stress and inflammation to initiate the chain of events of eosinopenia. Although returning to prior levels in the controls, the marrow events would be maintained by the pyelonephritis. The first marrow samples, 72 h after laparotomy, would thereby be well into the depressive phase of marrow activity.

The cure of the pneumococcal abscess with penicillin demonstrated that the mechanisms involved in the production of eosinopenia of acute inflammation are rapidly reversible with the cessation of the acute inflammatory response. It is noteworthy that there was no suggestion of eosinophil destruction during any of the experiments of this study nor in histological studies of the effects of acute infection on the eosinophilic granuloma, to be reported at a later time.

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