MECHANISM OF EOSINOPHILIA

I. Factors Affecting the Eosinophil Response of Rats to Trichinella Spiralis*

By ANTONY BASTEN‡, M.B., MARKLEY H. BOYER,§ M.D. and PAUL B. BEESON, M.D.

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

(Received for publication 10 December 1969)

Eosinophilia is a characteristic of most parasitic infestations, as well as various other disorders including dermatoses, allergies, polyarteritis nodosa, and some neoplastic diseases. The pathogenesis of this phenomenon has not been elucidated; furthermore there is as yet little evidence for a specific function for eosinophils (1). These cells resemble neutrophils in form, motility, and phagocytic capacity, and their granules contain many of the same enzymes, but they behave differently in response to infection, antigenic challenge, and adrenal steroid administration, as well as in their tendency to accumulate beneath surfaces exposed to the environment such as skin, bronchus, and gut.

We report here a study of the eosinophilia which accompanies trichinosis in rats. The intent of the experiments was to obtain information about the means by which the presence of parasites in other parts of the body stimulates increased production of eosinophils by hematopoietic tissue.

Materials and Methods

Animals.—Two strains of outbred parasite-free rats were employed; one, a Wistar strain, was obtained from the Medical Research Council Radiobiological Research Unit, Harwell, U.K.; the other, a fully SPF[1] Sprague-Dawley strain, came from the Scientific Products Farm, Ash, Kent, U.K. Male animals weighing between 180 and 250 g were employed. They were kept in standard metal grid cages, fed on modified Oxoid diet 41B, and given tap water to drink. For a week before each experiment they were placed in a quiet room illuminated by

* Supported by a grant from the Medical Research Council.

‡ Holder of a Nuffield Dominions Trust Fellowship. Present address: Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia.

§ Present address: Lakeside Hospital, Case Western Reserve University, Cleveland, Ohio 44106.

[1] Specifically pathogen-free rats were Caesarean derived and maintained as the name implies in a pathogen-free unit at place of origin.

artificial light between 9 a.m. and 9 p.m. The temperature of the room was maintained between 21° and 24°C. Diurnal fluctuations in eosinophil levels occur in rats (2), but with the hours of light and darkness used it was found that the blood levels showed minimal variation between 11 a.m. and 3:30 p.m. Therefore blood samples for counting were nearly all taken within this time period.

Trichinella Spiralis.—The Culbertson strain of the nematode *Trichinella spiralis* was obtained from Professor G. S. Nelson, London School of Hygiene and Tropical Medicine, and maintained by reinfestation in our colony. The life cycle commences when infested meat containing encysted larvae reaches the stomach of the new host. Peptic digestion liberates the larvae which burrow into the mucosa of the upper small intestine. There they develop into sexually mature forms which copulate by the end of the 2nd day. Subsequently the males die and are excreted but the gravid females persist. From about the 6th day a new generation of minute larvae is deposited within the mucosa whence migration to the blood stream takes place by way of regional lmphatic channels and the thoracic duct. Because of their small size (0.015 × 0.15 mm) young larvae can pass through the pulmonary circulation to all tissues of the host including heart, meninges, liver, lung and skeletal muscle, but they survive only in the last site. Here they attain full size (0.035 × 1.0 mm), become encapsulated, and can live for years. The life cycle is repeated when a new host ingests infested muscle.

Muscle-phase larvae for experimental purposes were obtained by the method described by Kagan (3), from rats which had been inoculated orally 4–6 wk previously. These were killed and their carcasses digested by means of an artificial gastric juice.[2] Larvae recovered in this way were washed three to four times in distilled water and four to six times in sterile isotonic saline before use. Estimation of the number of larval forms in a sample was usually carried out by permitting washed parasites to sediment in a graduated conical 10 ml test tube. A volume of 0.1 ml was found to contain 50,000 settled larvae ±10%. If more accurate quantitation was required a procedure similar to that described by Culbertson (4) was employed: larvae were suspended in nutrient broth containing 1.5% gelatin w/v and counted on a grid under the dissecting microscope. In this medium they retained full infectivity for a least 6 hr at room temperature and remained in relatively stable suspension for 1 min or 2, which facilitated administration by injection from a syringe.

To inoculate via the gastrointestinal tract a polythene tube was passed into the stomach of an animal anesthetized with ether and then a measured portion of larvae injected, using a tuberculin syringe. For parenteral administration the desired quantity of parasites was suspended in "single strength" Medium 199 (Grand Island Biological Co., Grand Island, N. Y.), thoroughly mixed by means of a Pasteur pipette, drawn immediately into a tuberculin syringe, and injected through a 23 gauge needle, with the syringe held near the vertical position.

Homogenization of Larvae.—Washed larvae suspended in Medium 199 were homogenized manually in a glass tissue grinder. Before injection a sample was examined microscopically to ensure that satisfactory fragmentation had been achieved.

Estimation of Hemagglutinin Response.—Specimens of blood for assay were obtained from the tail vein of anesthetized rats. The sera were separated and stored at −20°C. Before use they were heated to 56°C for 30 min to inactivate complement.

Antigen was prepared from a saline extract of larvae according to the method of Kagan (3). The antibody response was measured by a modification of the micromethod of Stavitsky (5). Formalinized "control" sheep cells (Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.) were washed in saline and coated with 1:20,000 tannic acid solution by incubation at 37°C for 15 min. Sensitization was carried out by mixing equal volumes of a suspension of tanned

[2] 1 liter contained 7 ml concentrated HCl and 5 g crystalline hog pepsin in distilled water.

cells and a 1:15 antigen solution. Antibody titers were assayed in plastic agglutination trays with round bottom cups (Flow Laboratories Irvine, Scotland), using 1:250 rabbit serum saline (pH 7.2) as diluent. The end point was taken as the maximum dilution showing obvious hemagglutination after standing at room temperature 12–14 hr.

Eosinophil Counts.—Blood for eosinophil counts was obtained from the lateral tail vein, with the animal under ether anesthesia. A 23 gauge needle was used to puncture the vein; after the first two drops had been discarded sufficient blood was collected on a small square of waxed paper (Parafilm) to fill a standard white cell hemocytometer pipette. Absolute eosinophil counts were made using Discombe's diluting fluid.[3] The diluent was made each week and filtered if necessary before use. Cell counts were performed in "bright line" counting chambers with modified Neubauer ruling.

Bone marrow was obtained by puncture of the femur. By using the method of Cameron and Watson (6) it was possible to obtain as many as three to four marrow samples from the same animal over a period of some days. Air-dried smears were fixed in methanol and stained with Leishman fluid.

Differential Leukocyte Response.—Total leukocyte counts were carried out using a standard diluting fluid of 2% glacial acetic acid and expressed as cells/mm^3 whole blood. Differential counts were estimated on the basis of 100 cells in smears stained with Leishman fluid.

Portal Vein Injection.—The abdomen was opened in the midline to expose the ventral surface of the liver. A loop of silk thread was passed under the portal vein to prevent retrograde flow, and a measured quantity of larvae suspended in medium 199 was injected proximally through a 23 gauge needle. The hole in the vein was sealed with methyl 2-cyanoacrylate adhesive (Ethicon, Inc., Somerville, N. J.).

Statistical Analysis.—The probability values (P) referred to in this and subsequent articles were calculated by means of Student's t test. When two sets of eosinophil responses were to be compared statistically each set of data was expressed in two ways: in the first a mean was calculated for the sum of the maximal eosinophil counts obtained by each animal during the period of eosinophilia, e.g. between the 4th and 8th days in Fig. 4; this provided a simple measure of the height of the response. In the second the mean of the individual eosinophil counts on each day of the same period, i.e. day 4 to day 8 in the example, was also obtained and a mean of the sum of these means calculated. In this way an estimate of the duration as well as the height of the eosinophil response was obtained. A signifiant difference was accepted only when both P values were less than 0.05. In the majority of instances P peak <0.05 was an adequate test of significance but occasionally P mean of means proved to be >0.05 when P peak was <0.05. Hence all P values referred to in tables and figures were caculated from comparison of the mean of the sum of the means.

RESULTS

Normal Range of Blood Eosinophil Counts.—Normal range (mean ± 2 SD) was established for the two strains of animal under study (Table I). Several strains of rats widely employed in experimental work proved unsatisfactory, because of marked variability in eosinophil counts, due presumably to pre-existing parasitic infestations (7). In contrast the strains employed here which had been reared under parasite-free conditions exhibited low and relatively stable blood levels as illustrated in Table I.

[3] Diluting fluid consisted of 5 volumes of 1% aqueous eosin Y, 5 volumes of acetone, and 90 volumes of distilled water.

Eosinophil Response to Inoculation by the Gastrointestinal Route.—Fig. 1 depicts the mean eosinophil and antibody responses of a representative group of rats following oral inoculation with 15 larvae/g body weight. In this as in subsequent figures the normal range is indicated by the cross-hatched area across the lower part of the graph. Eosinophilia was manifest by the 9th day, rising to a peak of 730 cells/mm^3 between the 11th and 15th day after challenge and then falling slightly to a plateau which was maintained throughout the remainder of the period of observation. Larger inocula produced higher peak responses, until they reached near lethal proportions. It will be noted that the maximal eosinophil response took place before the appearance of detectable hemagglutinating antibody.

When the eosinophil response of the same group of rats was followed for a total of 32 wk, a gradual decline in levels was noted (Fig. 2) despite persistence of parasites in the muscles of host animals. If the eosinophil responses of individual members of this group were plotted separately (Fig. 3) it was apparent that the number of cells in peripheral blood fluctuated markedly from week to week. After the initial peak at about 2 wk one or more subsequent rises occurred in 12 of 13 animals followed for that period of time. On the other hand, antibody levels did not seem to fluctuate.

TABLE I

Normal Ranges of Blood Eosinophil Counts

Strain	Number	Mean count/mm^3 and SD	Normal range*
Wistar	282	80 ± 40	0–160
Sprague-Dawley	143	102 ± 42	18–186

* Mean±2 standard deviations (SD).

Eosinophil counts were performed between 11 a.m. and 3.30 p.m. when diurnal variation was minimal.

The bone marrow response to gastrointestinal trichinosis is presented in Table II. Serial specimens of blood and marrow were collected from a group of rats before inoculation with larvae and afterwards on the 15th and 28th days. The number of recognizable eosinophils/1000 nucleated cells was counted in each of three smears and the sum of the means recorded. At the time of maximal eosinophilia (day 15) as indicated by a 10-fold rise in blood level, there was a 4-fold increase in recognizable eosinophils in bone marrow, many of which were at the myelocytic stage.

Eosinophil Response to Inoculation by the Intravenous Route.—As a standard method of challenge the gastrointestinal form of inoculation had two disadvantages: first, approximately 2 wk elapsed between inoculation and development of the peak eosinophil response; second, the persistence of living parasites and

the countinuing stimulus of their growth complicated analysis of the resulting response, especially in later experiments designed to evaluate eosinophilia as a manifestation of immunity. Muscle-stage larvae were therefore administered by the intravenous route in the hope of obtaining a simpler and briefer experimental procedure. After injection the parasites embolized to the lungs where they were trapped because of their size. Respiratory distress only became evident when the size of inoculation exceeded 20,000. In Fig. 4 the eosinophil response

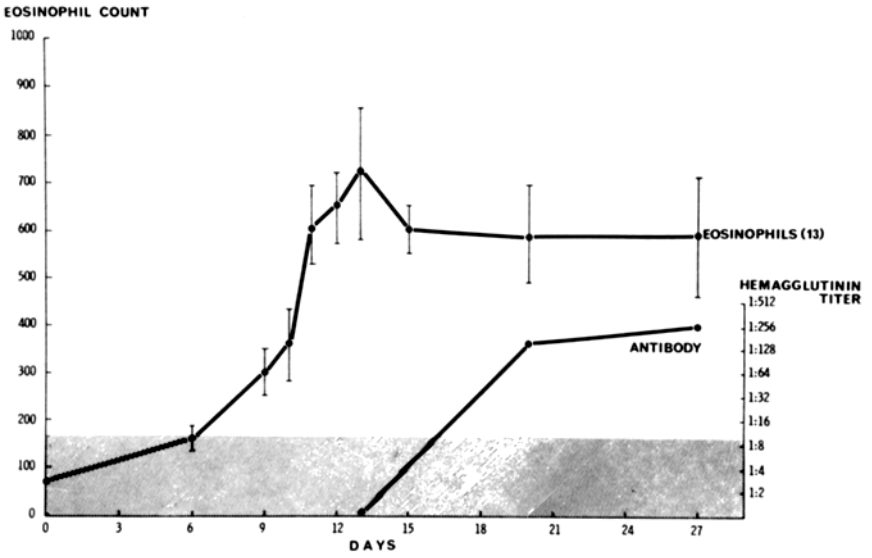

FIG. 1. Eosinophil and antibody responses to inoculation of *Trichinella* larvae by the gastrointestinal route. Animals were given 15 larvae per g of body weight. The term eosinophil count refers to the number of blood eosinophils/mm^3. Each point represents the mean of observations recorded at a particular time. Variations about the mean are expressed as ± 1 standard error (SE). The number of test animals is shown at the end of each graph in brackets. Cross-hatching indicates the normal range (mean ± 2 SD) of eosinophil counts for the strain of rat employed.

to injection of 10,000 larvae has been compared with that following an injection of saline. For the first 48 hr the blood level remained within the normal range but then the counts in animals given larvae rose rapidly to reach a mean peak value of approximately 650 cells/mm^3 on the 6th day. Thereafter the counts fell back to the normal range by the 14th day. Of interest is the fact that secondary waves of eosinophilia did not occur when this route of inoculation was employed. In contrast to the response to oral infestation free antibody was detected in serum at the time of maximal eosinophilia, the mean hemagglutinin titer rising to a peak of 1:32 on the 8th day. At the termination of the experiment carcasses were digested in artificial gastric juice; no intact larvae were found in

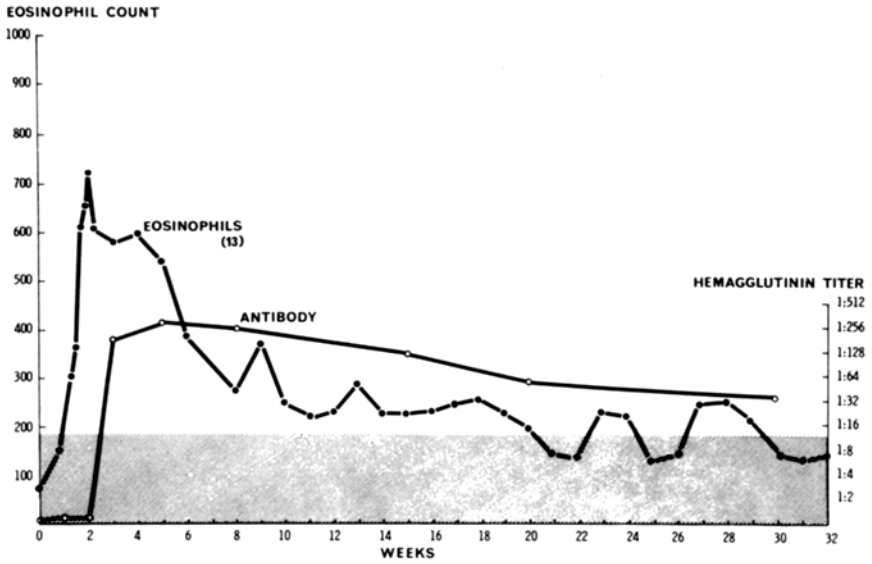

FIG. 2. Eosinophil and antibody responses of a group of rats followed for 32 wk after inoculation of *Trichinella* larvae by the gastrointestinal route.

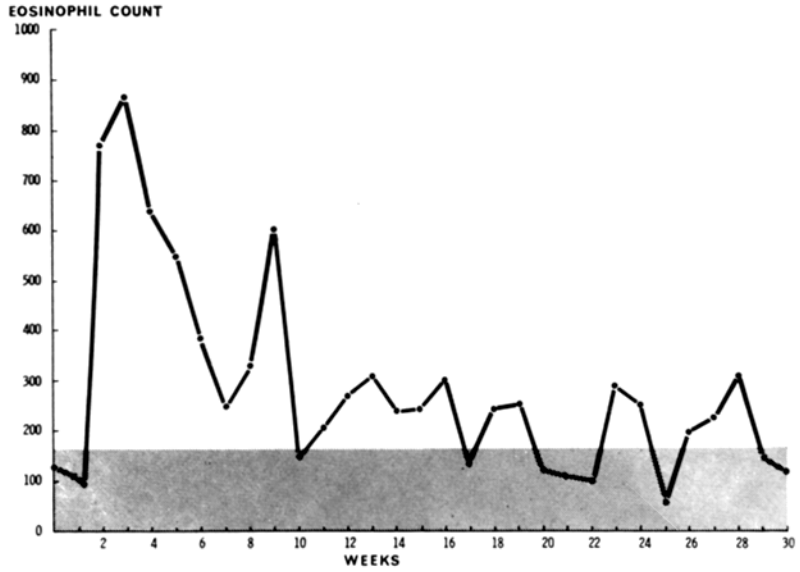

FIG. 3. Blood eosinophil level of an individual rat followed for 30 wk after infestation.

the lungs of any recipients, and of the 25 rats studied 23 had none in their skeletal musculature, while the muscles of the remaining two contained fewer than 100 larvae/rat.

TABLE II

Eosinophil Levels in Blood and Bone Marrow

Time after infestation	Blood	Bone marrow
days	*Mean eosinophil count/mm³*	*Mean eosinophils/1000 nucleated cells*
0	90	29
15	1290	125
28	825	91

These figures were obtained from simultaneous estimations of blood and bone marrow in each of 10 animals during the course of trichinosis. *Trichinella* larvae were deposited in the stomach on day 0.

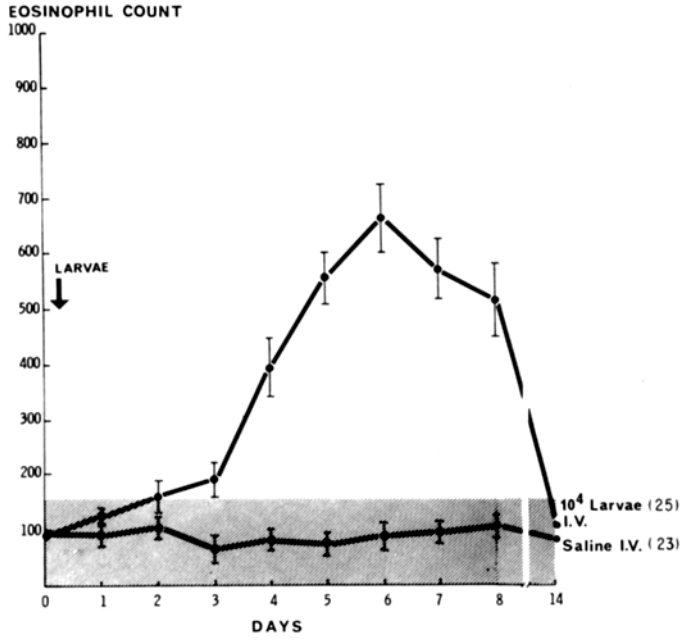

FIG. 4. Eosinophil response to inoculation of *Trichinella* larvae by the intravenous route. The broad arrow indicates the time at which the intravenous injection was given.

Some other rats were sacrificed at intervals after inoculation. Within hours of lodgment of larvae in the pulmonary circulation the lungs showed the macroscopic features of an acute widespread hemorrhagic pneumonia. Microscopically there was evidence of local destruction of injected parasites within 24 hr, at

which time they were surrounded by neutrophils, eosinophils, small lymphocytes, and a dense accumulation of mononuclear cells resembling macrophages. A detailed histological study of this lung lesion is now being carried out.

TABLE III

Eosinophil and Antibody Responses to Intravenous Injection of Various Forms of Parasitic Material

Number of rats	Number of larvae	Type of inoculum	Day 0	Peak response (days 4-8)	Hemagglutinin titer (day 54)
			Mean eosinophil counts/mm³ ± SE		
23	None	Saline	95 ± 7.5	147 ± 13.3	<1:2
12	5,000	Viable larvae	40 ± 8.6	627 ± 59*	<1:2
25	10,000	Viable larvae	79 ± 9.7	780 ± 85*	1:16
10	10,000	Nonviable larvae	94 ± 17	530 ± 95*	Not done

* A significant difference ($P < 0.05$).

Each response has been compared with that of the saline treated controls. The term "peak response" refers to the mean of the highest eosinophil counts attained in each group during the specified period. Note that the eosinophil response to 5000 larvae developed in the absence of a detectable hemagglutinin titer.

TABLE IV

Total and Differential Leukocyte Responses to Inoculation with Trichinella Larvae

Trichinella larvae given by gastrointestinal route					*Trichinella* larvae given intravenously				
Days after inoculation	Mean leukocyte counts/mm³				Days after inoculation				
	Total	Neutrophil	Eosinophil	Lymphocyte		Total	Neutrophil	Eosinophil	Lymphocyte
						Mean leukocyte counts/mm³			
0	11,450	2465	80	8760	0	16,800	4450	125	10,350
6	12,810	2730	30	9805	4	23,615	4900	625	18,615
10	18,090	5075	110	12,170	5	25,015	6985	660	18,265
13	20,390	6650	295	12,875	6	30,585	7530	1260	21,765
15	20,250	5390	535	13,765	7	30,500	6065	870	22,560
20	11,720	2790	310	8410	8	34,835	7820	745	26,435

Animals inoculated by the gastrointestinal route were given 15 larvae per g of body weight; those challenged intravenously received 10,000 parasites each.

Each figure represents the mean of counts on six rats.

When half the number of larvae, i.e. 5000, was used, or the parasites were inactivated by freezing and thawing before injection (with loss of motility and reproductive capacity), a smaller eosinophilia ensued with earlier return to the normal range (Table III). The eosinophil response to 5000 larvae developed in the absence of detectable hemagglutinating antibody.

Another group of rats was given 5000 parasites intravenously on two occasions 21 days apart. A comparison of the two responses showed that the peak

reaction to the second intravenous injection was significantly greater than that of the first challenge, both in height and magnitude. This subject will be considered in more detail in subsequent articles.

Differential Leukocyte Response.—Table IV shows the total and differential leukocyte responses of groups of rats inoculated with larvae by both gastrointestinal and intravenous routes. The results indicate that eosinophilia was accompanied by moderate increases in both neutrophil leucocytes and mononuclear cells.

Lack of Eosinophil Response to Homogenates, Crude Chemical Fractions, or Metabolic Products of Parasites

Homogenates.—Trichinella larvae whether living or dead were shown to be capable of inducing a substantial eosinophilia following intravenous injection and lodgment in the lung. It was important to test whether development of this eosinophil response was dependent on the resultant pulmonary lesion. Larvae were therefore ground into fragments small enough to pass through the pulmonary circulation.

A standard suspension of 10,000 larvae/ml was divided into two parts, one being homgenized before intravenous injection. 1 ml of homogenate suspension was given to each of six Sprague-Dawley rats while a further six animals received the same quantity of intact larvae.

The mean eosinophil and antibody responses are shown in Fig. 5. Rats given intact larvae developed eosinophilia whereas recipients of homogenate failed to do so. On the other hand the antibody response to homogenate appeared to be greater; on day 4, for instance, no antibody was detected in rats which had received intact larvae, whereas recipients of homogenized parasites showed a significant hemagglutinin response.

To confirm that homogenized larvae were capable of passing through the pulmonary circulation homogenate was injected as before, but recipients were killed 10 min later and the lungs removed for histological examination. Microscopy indicated that little or no parasitic material remained in pulmonary tissue.

Further analysis of the effect of larval fragmentation was then carried out.

After homogenization in a tissue grinder parasites were subjected to additional disruption by intermittent exposure to an ultrasonic disintegrator for periods of up to 30 sec at 4°C. The resultant suspension was centrifuged at 20,000 *g* for 10 min and both the supernatant and precipitate assayed for eosinophilic activity.

Groups of three animals were each given samples derived from 1000, 10,000, and 100,000 intact larvae but none of them developed an eosinophil response.

Chemical Extracts.—Approximately 2 g fresh or frozen larvae were homogenized in liquid nitrogen to form a fine powder which was extracted with 1 ml of 30% perchloric acid. Following addition of 5 ml of distilled water the mixture was homogenized with a glass pestle driven

by a low-speed motor and centrifuged at 20,000 *g* for 10 min. The supernatant solution was pipetted off, neutralized with 20% potassium hydroxide, and permitted to stand for 30 min in the cold. The precipitate of potassium perchlorate was removed by centrifugation leaving a soluble deproteinized extract containing low molecular weight substances including peptides and polysaccharides. The pH of the extract was adjusted to neutrality before injection into recipient animals.

A second extract was prepared by homogenizing approximately 2 g larvae in liquid nitrogen as described previously, but this time extraction was performed using 5 ml of acid alcohol

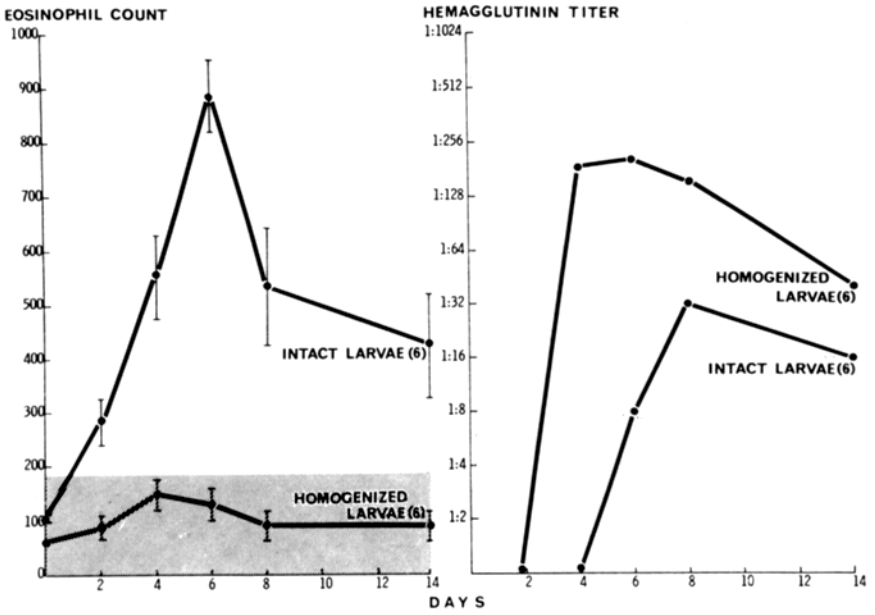

FIG. 5. Eosinophil and antibody responses to intravenous inoculation of intact and homogenized *Trichinella* larvae.

The resultant mixture was centrifuged at 20,000 *g* for 10 min at 4°C and the supernatant removed. The residue was then reextracted with acid alcohol and the supernatant from each extraction pooled. To the combined extracts 10 volumes of acetone were added resulting in formation of a white precipitate which was collected 1 hr later by centrifugation. The precipitate was dried *in vacuo* at 4°C and redissolved in saline before assay.

Groups of four animals were each given samples derived from 1000, 10,000, and 100,000 intact larvae but again none of them developed eosinophilia.

Metabolic Products.—Large numbers of parasites were obtained from infested carcasses by peptic digestion, washed thoroughly in Medium 199 containing antibiotics, and cultured in vitro for 5 days according to the method used by Denham (8) in his study of antigenic products. Afterwards the supernatant fluid, rich in larval metabolites, was filtered, centrifuged at 20,000 *g* for 10 min at 4°C, and the sediment was discarded.

The extract was assayed by inoculation of three groups of normal animals. Group I (four rats) received a single intraperitoneal injection of 5 ml. Group 2 (22 rats) was given a total of 25–50 ml in divided doses intraperitoneally. Group 3 (four rats) received 50 ml in the form of a continuous intravenous infusion kept at 4°C. No inocula proved capable of inducing a significant eosinophil response.

Eosinophil Response to Inoculation of Larvae by Various Parenteral Routes.—In Table V the eosinophil responses to larval administration in various sites are compared with the "standard" reaction induced by giving 10,000

TABLE V

Eosinophil and Antibody Responses to Inoculation of Intact Trichinella Larvae by Different Routes

Number of rats	Number of larvae injected	Route of infection	Day 0	Peak response (days 4–8)	Hemagglutinin titer (day 54)
			Mean eosinophil counts/mm³ ± SE		
23	Saline only	Tail vein	95 ± 7.5	147 ± 13	<1:2
25	10,000	Tail vein	79 ± 9.5	780 ± 85*	1:16
7	10,000	Intraperitoneal	72 ± 10	186 ± 19	1:2
4	50,000	Intraperitoneal	94 ± 14.5	402 ± 62*	Not done
5	10,000	Subcutaneous	67 ± 14	134 ± 38	<1:2
5	50,000	Subcutaneous	78 ± 11.5	176 ± 26	Not done
5	10,000	Intramuscular	81 ± 10.5	119 ± 8	<1:2
5	10,000	Intraarterial	80 ± 16.5	423 ± 76*	1:4–1:8
8	10,000	Portal vein	63 ± 16	230 ± 38	<1:2

* A significant difference ($P < 0.05$).

Recipients of saline only and 10,000 larvae via a tail vein are included for comparison. Each response has been compared with that of the saline-treated controls. Hemagglutinin titers of <1:2 indicate no detectable antibody response.

larvae intravenously. It will be noted that a detectable rise in eosinophils did not result from injection of this number subcutaneously, intramuscularly, or intraperitoneally. When the size of larval inoculum was increased to 50,000 there was a modest but statistically significant response to intraperitoneal injection, but still none to subcutaneous injection. When 10,000 larvae were introduced into the portal vein the eosinophil level appeared to rise but its peak value was not significantly elevated above that of the saline controls. On the other hand, injection into the abdominal aorta gave a significant reaction though not as great as when larvae were inoculated via the caval system.

Role of Spleen in the Eosinophil Response.—Earlier it was shown that homogenized larvae were incapable of inducing an eosinophilia. This effect was thought to be attributable to processing of parasitic material in reticulo-endothelial tissues. It therefore became of interest to examine the role of the spleen in the eosinophil response.

Splenectomy was performed on eight rats under ether anesthesia through a left subcostal incision. 48 hr before operation each animal had received an intravenous injection of neoarsphenamine 2 mg/100 g body weight to prevent activation of possible bartonellosis (9). 24 hr after surgery four animals (Group I) were inoculated with 10,000 larvae intravenously and the remainder (Group II) received saline only.

The eosinophil and differential leukocyte responses are shown in Tables VI and VII. As expected from other work (10) splenectomy resulted in some in-

TABLE VI

Effect of Splenectomy on the Total and Differential Leukocyte Counts

Total counts		Neutrophil		Eosinophi		Lymphocyte	
			Day				
−1	+6	−1	+6	−1	+6	−1	+6
Mean leukocyte counts/mm³							
7025	17,675	415	5390	59	342	6360	12,165

Each figure represents the mean of counts on five rats.

TABLE VII

Effect of Splenectomy on the Eosinophil and Antibody Responses to Intravenous Inoculation of Trichinella Larvae

Operation (day −1)	Challenge (day 0)	Number	Day −1	Peak response (days 4–8)	Hemagglutinin titer (day +8)
			Mean eosinophil counts/mm³ ± SE		
Laparotomy	Saline	4	72 ± 9.5	158 ± 21	<1:2
Splenectomy	Saline	5	59 ± 12	380 ± 32.5	<1:2
Splenectomy	10,000 larvae	4	65 ± 15	1740 ± 67	1:4
None	10,000 larvae	25	80 ± 10	780 ± 85	1:32

crease in the levels of neutrophils and lymphocytes, as well as eosinophils (see saline controls). Members of Group I which received parasites showed an average eosinophil count of 1740 cells/mm³ on the 6th day. This should be compared with the 6th day value of 667 cells/mm³ obtained by inoculation of non-splenectomized rats with the same quantity of larvae. Antibody production after splenectomy was low, reaching a titer of only 1:4 on day 8.

DISCUSSION

The objective of this investigation was to obtain information about the mechanism of eosinophilia; we were not primarily investigating either the function of these cells or factors such as histamine that are alleged to cause them to accumulate in tissues. Trichinosis in rats was selected as the test system because it is easy to handle in the laboratory and because much is already

known about its characteristics in experimental animals. Quantitation of results by means of blood counts has the advantages of accuracy, simplicity, and repeated assay in one test animal.

The assumption underlying this work is that a rise in number of circulating eosinophils some days after inoculation of parasitic material can be regarded as an indicator of increased eosinophil production in hemopoietic tissues. The mature eosinophil is thought to be an end-cell, incapable of further cell division; it develops in bone marrow, circulates for a few hours in the blood (11) before migrating into extravascular sites where it dies (or is shed from a mucosal surface). It does not recirculate in the blood. Good reasons can be given to support the assumption that a sustained increase in number of cells in the circulation, occurring some days after a stimulus, reflects increased production. Several workers have found clear evidence of increased eosinopoiesis in the marrow during the invasive stage of trichinosis. Opie (12) who studied the disease in guinea pigs, described the marrow thus: "Cells with eosinophil granulation are present in immense number, and particularly numerous are the eosinophil myelocytes, cells peculiar to the bone marrow." The interval of some days before blood eosinophilia appears is also consistent with increased production. Kinetic studies using pulse-labeling with tritiated thymidine show a lag of 30 hr or more before the first labeled cells appear in the circulation (13). Spry, in our laboratories (C.J.F. Spry, 1969, personal communication), has used this technique to monitor eosinophil kinetics during trichinosis. He has confirmed other work on the time lag before first appearance, and his data show no evidence of increased life span in the circulation. On the contrary, they indicate an accelerated rate of removal from the blood stream during the early phases of the response. The time of onset of the eosinophilia observed in our studies when two forms of challenge were employed is also in keeping with this viewpoint. With "natural" infestation by the gastrointestinal route the time required for maturation and reproduction of parasites is 5 or 6 days. Under these circumstances eosinophilia was first noted about the 9th day and reached its peak between the 11th and 15th days. On the other hand, when muscle-stage larvae were injected intravenously a rise began as early as the 2nd day and reached its peak between the 4th and 8th days.

We recognize, of course, that circulatory eosinophilia does not provide a direct quantitative correlation with bone marrow production, since an accompanying increased rate of cell migration out of the circulation almost certainly occurs. The total number of eosinophils in the blood represents only a minute fraction of the total body content. Rytömaa (7) estimated that for every circulating eosinophil there are 200–300 mature cells in the bone marrow reserve, as well as a similar number in extravascular tissue. Obviously then, there are effective homeostatic mechanisms which operate to prevent accumulation of eosinophils in the circulation. Our data bear this out, in that a 4-fold increase

in the relatively large stores of cells in bone marrow was reflected by only a 10-fold increase in number of circulating cells. It is reasonable to assume that a significant elevation in eosinophil level some days after a challenge reflects substantial traffic of these cells between sites of origin and disposal.

Surprisingly little use has been made of daily blood levels in studies of the eosinophil. Doubtless some investigators have regarded blood levels as too variable for reliance, because of the well-known fluctuations due to diurnal rhythm, stress, and the relatively large number of mature cells in other body compartments. We have found it possible to cope with the variation due to diurnal changes and stress, by the precautions already outlined and by the use of suitable controls. Changes in blood level due to shifts between compartments, well documented in Hudson's studies (14) do not persist as long as 48 hr. For experiments extending over periods of several days, the essential requirement, in our view, is to employ strains of experimental animals which exhibit low, stable eosinophil levels. It is likely that undetected parasitic infestation in many commonly used strains accounts for erratic fluctuations in blood level, such as were encountered in our long-term observations showing waves of eosinophilia months after induction of trichinosis. Several strains of rats tested in the course of this work proved unsuitable, because of such fluctuations, their counts ranging between 150 and 600/mm^3 from day to day.

The erratic waves of eosinophilia which occur for months after *Trichinella* infestation, long after the parasites have ceased to circulate and are confined "quietly" to skeletal muscle, are difficult to explain. Similar observations have been made by others in rats (15), in man (16), and in responses to stimuli other than trichinosis (17, 18). The fact that these secondary waves did not occur in animals challenged intravenously suggests that the fluctuations are related to the presence of living parasites.

As a test system for study of the eosinophil response the intravenous injection of muscle-stage larvae has advantages of brevity and remarkable reproducibility. Inoculation by this route results in an acute inflammatory reaction in the lungs within hours and a peak circulating eosinophil level in a week. One point clearly established by this procedure is that vigorous eosinopoiesis can result from a single exposure to foreign matter. Most previous work on eosinophilia has involved the continuous stimulus of active infestation, or the repeated administration of nonliving antigens.

Of particular interest were the findings when larval fragments were injected intravenously. Although a vigorous eosinophilia had been evoked by injection of whole larvae, living or dead, the simple procedure of crude homogenization, reducing the parasitic tissue to particles small enough to pass through the pulmonary vascular bed, abolished the eosinophil response. This argued against the existence of some unique eosinopoietic constituent of the parasites and placed emphasis on the local cellular reaction to the foreign matter. The in-

ability of chemical fractions or metabolic products of the parasite to initiate eosinophilia provides additional support for this line of reasoning.

Further clues to the pathogenesis of the eosinophil reaction are provided by the experiments in which muscle-stage larvae were administered by various parenteral routes. Thus, whereas inoculation of 5000 or 10,000 larvae intravenously elicited a sharp eosinophil response, the administration of 10,000 intraperitoneally or subcutaneously failed to produce any rise, and even 5 times as many parasites caused only a modest eosinophilia in the former and failed to elicit a detectable response in the latter. Since eosinophilia is often associated clinically with pulmonary disorders the question arose whether some component of the lung played a part in the reaction to injection via a tail vein, but this possibility seemed unlikely when it was found that a significant response resulted from injection of larvae into the abdominal aorta. We tend to infer that the eosinophil reaction depends in part on the kind and number of cells which come into contact with the material in different anatomic sites. The greater response to intravascular deposition may be related to the widespread distribution of individual parasites, permitting quick access of bloodborne and tissue cells in the innumerable small foci of acute inflammation.

Several of the results described in this article are in keeping with the possibility that enhanced eosinopoiesis represents one form of immunologic response. First, a period of some days intervened between introduction of the foreign material and the observed reaction. Second, there was a heightened response to a later challenge. Third, the reaction to administration of larvae via the portal vein was diminished, as has been found to characterize certain other types of immune reaction (19, 20).

Special attention was given to the relation between eosinophilia and antibody formation, but, as measured by the hemagglutination technique with whole larval substance as antigen, there was little to suggest interdependence. When the gastrointestinal route of inoculation was employed the eosinophil response developed and passed its peak before antibody could be demonstrated. Using the intravenous method of challenge, with 5000 larvae there was a sharp eosinophil reaction, yet circulating antibody could not be found. When the number of larvae was increased to 10,000 both responses became evident at about the same time, although the antibodies persisted long after the eosinophil level had returned to normal. When fragmented larvae were injected intravenously a prompt and substantial antibody response ensued, but there was no eosinophilia. In splenectomized test animals the eosinophil reaction was augmented and the antibody response was reduced. It is reasonable to conclude that the eosinophil response shows no correlation with the level of hemagglutinating antibody, but it must be conceded that other kinds of antibody than were detected could play a part. We have not made an exhaustive effort to demonstrate a correlation between eosinophilia and some

other form of antibody, mainly because later experiments indicated that the stimulus to eosinopoiesis is mediated by cells.

SUMMARY

The phenomenon of eosinophilia was studied in rats using inoculation with *Trichinella* larvae as the experimental stimulus. Comparisons were made between the eosinophil response accompanying active infestation via the gastrointestinal tract and that resulting from parenteral inoculation of larvae or their products.

A vigorous eosinophilia could be provoked by a single intravenous injection of intact parasites. In this circumstance the larvae lodged in the lungs causing an acute inflammatory reaction which led to their disintegration within 24 hr. Intraaortic injection also produced a significant response, whereas inoculation of the same number of parasites by the intramuscular, intraperitoneal, or subcutaneous routes did not cause eosinophilia. Eosinophilia likewise failed to develop if parasites were homogenized before intravenous injection, so that they were not arrested in the lungs.

Antibody levels, as measured by a hemagglutination technique, using whole larval extract as antigen, did not correlate closely with the eosinophil response.

The findings are interpreted as suggesting that increased eosinophil production is induced under some circumstances as a consequence of interaction between intact parasites and certain host cells in blood and tissue. No evidence was found for the existence of a specific constituent of the parasite capable of stimulating eosinophil production.

Attention is directed to features of eosinophilia which fit with the concept that this phenomenon belongs in the category of immunologic reactions.

The authors are indebted to Dr. D. H. Williamson for help in the preparation of chemical extracts from *Trichinella* larvae.

BIBLIOGRAPHY

1. Hirsch, J. G. 1965. The eosinophil leucocyte. *In* The Inflammatory Process. B. W. Zweifach, L. Grant, and R. T. McCluskey, editors. Academic Press Inc., New York. 266.
2. Jakobson, T., and H. Hortlung. 1954. Studies on the endocrine regulation of the number of circulating eosinophil cells in the rat. *Acta Endocrinol.*, **15**:265.
3. Kagan, I. G. 1960. Trichinosis: a review of biological, serological and immunological aspects. *J. Infec. Dis.* **107**:65.
4. Culbertson, J. T. 1942. Active immunity in mice against *Trichinella spiralis*. *J. Parasitol.* **28**:197.
5. Stavitsky, A. B. 1954. Micromethods for the study of proteins and antibodies. I. Procedure and general applications of hemagglutination and hemagglutination-

inhibition reactions with tannic acid and protein-treated red blood cells. *J. Immunol.* **72**:360.

6. Cameron, D. G., and G. M. Watson. 1948. Femoral bone marrow biopsy in the albino rat. *Blood.* **3**:292.
7. Rytömaa, T. 1960. Organ distribution and histochemical properties of eosinophil granulocytes in rat. *Acta Pathol. Microbiol. Scand. Suppl.* **50**:1.
8. Denham, D. A. 1967. Applications of the in vitro culture of nematodes especially *Trichinella spiralis*. *In* Proceedings of the Fifth Symposium of the British Society for Parasitology. A. E. R. Taylor, editor. Blackwell Scientific Publications Ltd., Oxford, England. 49.
9. Sanders, A. G., and H. W. Florey. 1940. The effects of the removal of lymphoid tissue. *Brit. J. Exp. Pathol.* **21**:275.
10. Lipson, R. L., E. D. Bayrd, and C. H. Watkins. 1959. The post splenectomy blood picture. *Amer. J. Clin. Pathol.* **32**:526.
11. Hudson, G. 1968. Quantitative study of the eosinophil granulocytes. *Seminars Hematol.* **5**:166.
12. Opie, E. L. 1904. An experimental study of the relation of cells with eosinophil granulation to infection with an animal parasite (*Trichina spiralis*). *Amer. J. Med. Sci.* **127**:477.
13. Bro-Rasmussen, F., V. Andersen, and O. Henriksen. 1967. The kinetics of eosinophil granulocytes in rats. Autoradiographic studies. *Scand. J. Haematol.* **4**:81.
14. Hudson, G. 1963. Changes in the marrow reserve of eosinophils following re-exposure to foreign protein. *Brit. J. Haematol.* **9**:446.
15. Zaiman, H., and H. Villaverde. 1964. Studies on the eosinophil response of parabiotic rats infected with *Trichinella spiralis*. *Exp. Parasitol.* **15**:14.
16. Della Vida, B. L., and S. C. Dyke. 1941. Blood picture in trichiniasis. *Lancet.* **2**:69.
17. Rogers, B. O., J. M. Converse, C. A. Taylor, and R. M. Campbell. 1953. Eosinophils in human skin homografting. *Proc. Soc. Exp. Biol. Med.* **82**:523.
18. Weber, T. B., and R. Rubin. 1958. The eosinophilic response to infection with the cattle lung worm, *Dictyocaulus viviparus*. *J. Infec. Dis.* **102**:214.
19. Cantor, H. M., and A. E. Dumont. 1967. Hepatic suppression of sensitization to antigen absorbed into the portal system. *Nature (London).* **215**:744.
20. Dresser, D. W., and N. A. Mitchison, 1968. The mechanism of immunological paralysis. *Advan. Immunol.* **8**:129.