

EXPERIMENTAL EOSINOPHILIA: LOCAL TISSUE REACTION TO ASCARIS EXTRACTS

John Vaughn, M.D., Houston, Texas

THE EOSINOPHIL has a remarkably frequent, if not constant, association with the breakdown of protein. In the antigen-antibody reaction there is an accumulation of protein end products, some with far-reaching physiologic and pathologic potential.^{1, 2} Injections of polypeptides derived from parasitic worms, particularly *Ascaris*, can produce anaphylactoid reactions and eosinophilia in experimental animals, effects probably unrelated to the antigen-antibody reaction as they can be produced upon single injection. Attempts to isolate substances which can evoke experimental eosinophilia have usually yielded mixtures of unidentified polypeptides, polysaccharides, or their catabolic products, among which histamine is repeatedly mentioned.³⁻⁹

In two previous studies^{9, 10} water soluble polypeptides and polysaccharides derived from *Ascaris* were injected into the peritoneal cavity of guinea pigs. Readily absorbed, these materials appeared to excite little local tissue reaction but did produce interesting changes in remote tissues and organs which were regarded as part of the later phase of eosinophil activity. Insoluble *Ascaris* derivatives have excited local reactions,⁴ and, to facilitate study of the earlier phases of eosinophil activity, insoluble *Ascaris* materials were prepared and injected into the subcutaneous tissues of the guinea pig. The ensuing histologic changes and their probable relationship to the function of the eosinophil are presented here.

MATERIALS, ANIMALS, AND METHODS

Ascaris Extracts.—Washed *Ascaris lumbricoides* var. *suum*, obtained from slaughtered hogs, were pulped in a tissue blender in an equal volume of 10 per cent trichloroacetic acid, the resulting fluid quickly settling to a pink sediment with white supernatant. Polysaccharide was extracted from the supernatant as described elsewhere,⁹ while the sediment was washed in water, adjusted to pH 7.2, passed through ethanols and ether, and finally dried at room temperature. This was a gray-green granular material which when wet resembled the grumous contents of an epidermoid cyst. Both polysaccharide and protein rich material were sterilized by heating to 56° C. for 20 minutes on 3 successive

From the Department of Pathology, Baylor University College of Medicine, Houston, Texas. Supported by U. S. Public Health Service, National Institutes of Health Grant No. 2261. Received for publication June 13, 1961.

days; cheek cultures in thioglycollate broth were sterile at 72 hours' incubation. These materials, stored at -20°C ., were used within 4 weeks of preparation.

Animals.—Baby guinea pigs were separated from the mother at 7 days and tests were started before they were 4 weeks old; all weighed less than 200 grams. The diet was a standard pellet chow supplemented by fresh vegetables thrice weekly. Materials were inserted into the subcutaneous tissues through small wounds in the skin of the back, two implants being made in each animal, one sealed with collodion and the other left uncovered. This latter was induced at intervals to exude fluid by gently squeezing the tissues; smears were made of the exudate using a technique similar to that of Kolouch.¹¹ The method of Rebuck and his co-workers,¹² in which coverglasses are placed over the wound and removed at intervals, was unsuccessful because of loss, breakage, and spoilage of the specimens by the animals. Blood samples were taken from a freshly nicked ear and animals were killed by exposure to chloroform vapor, autopsied at once, and appropriate specimens were taken for histologic and hematologic study.

Methods.—Standard techniques were used for eosinophil and total leukocyte counts. Smears were made on slides, blood being spread by a coverglass pulled sideways, so combining the advantage of more uniform distribution of cells in the coverglass method¹³ with the ease of staining slides. Stains used for blood smears were Wright and Giemsa, and for tissues were hematoxylin-eosin, trichrome, and Giemsa.

Because of the many inherent errors in counting blood cells (even by practiced technique), direct and indirect counts of eosinophils frequently failed to agree, but the difference was seldom greater than ± 20 per cent. All eosinophil counts in the tables are expressed to the nearest ten cells per cubic millimeter, a degree of significance adequate for this study. In assessing other leukocyte changes, all white cells were computed in absolute numbers from total count and smear ratios; no comparisons were made on percentages alone. These data proved to be voluminous and, as they added little to this presentation, they are not reported here. Histologically there was little difference between sealed and open lesions. To avoid repetitive and possibly confusing detail, the lesions have not been described individually. Instead, a collective assessment of lesions in chronological sequence is given.

PROCEDURE

Experiment 1.—

Each of 15 guinea pigs received two subcutaneous implantations of *Ascaris* polysaccharide (0.25 mg.), one wound being sealed and the other left uncovered. In the ensuing 72 hours, samples of peripheral blood and exudate from the uncovered wound were taken at intervals. The animals were killed at 1, 2, 3, 6, 9, 12, 15, 24, 48, and 72 hours and autopsied. Control peripheral blood studies were made on guinea pigs subjected to incision without implantation.

Blood changes: Nine animals showed a mild increase in neutrophils which appeared 2 hours after implantation, reached a peak of 10,000 to 12,000 leukocytes per cubic millimeter at about 12 hours, and subsided to normal levels at 24 hours. There was a simultaneous fall in lymphocytes to low normal values, with a return to initial levels at 24 hours. With the exception of animals killed before 3 hours, all showed a distinct eosinophilia (Table I) maximal at 12 or 24 hours and subsiding at 48 hours. Monocytes showed no consistent trend and no change in basophils was noted. Control studies were unremarkable.

TABLE I. EOSINOPHIL LEVELS IN NORMAL UNSENSITIZED GUINEA PIGS GIVEN SUBCUTANEOUS IMPLANTS OF ASCARIS POLYSACCHARIDE, EXPRESSED AS CELLS PER CUBIC MILLIMETER OF BLOOD

	INITIAL	HOURS								
		1	2	3	6	12	24	36	48	72
1	250	240	—	—	—	—	—	—	—	—
2	90	180	—	—	—	—	—	—	—	—
3	230	—	590	—	—	—	—	—	—	—
4	170	—	270	—	—	—	—	—	—	—
5	110	270	520	1,830	—	—	—	—	—	—
6	120	100	290	720	—	—	—	—	—	—
7	30	—	—	180	500	—	—	—	—	—
8	390	—	—	420	490	—	—	—	—	—
9	120	—	—	—	400	1,120	—	—	—	—
10	180	—	—	—	890	780	—	—	—	—
11	60	—	220	—	1,100	1,600	2,200	—	—	—
12	220	—	250	500	610	720	780	—	—	—
13	170	—	—	—	960	—	1,910	1,200	—	—
14	210	—	—	600	—	420	350	—	270	—
15	130	—	—	250	—	450	270	—	260	180
Control	190	60	—	180	0	70	110	—	200	180
Control	120	—	60	—	80	150	210	—	120	160

Legend: The symbol—indicates that no count was made.

Exudates: Early smears contained only cellular debris and a few red cells but at 3 hours became more cellular with granulocytes and monocytes prominent. In succeeding smears, granulocytes increased steadily in numbers with a left nuclear shift, while monocytes became less conspicuous and later disappeared. Eosinophils were scanty and no basophils, lymphocytes, or plasma cells were seen; occasional histiocytes appeared in late smears. Smears of exudate taken at 24 hours yielded little cellular material and were discarded.

Skin lesions: Sealed and open lesions differed little, and all sections included a small epidermal wound extending into the upper dermis. In the earliest lesions, this wound contained red blood cells, fibrin, and a few leukocytes but neither polysaccharide nor any significant open spaces were found. At 3 hours, hyperemia of local capillaries and leukocytic margination with emigration were observed, the cells being neutrophils and monocytes. In later sections the neutrophilic infiltration increased steadily up to 24 hours after which it became noticeably less dense. Eosinophils were not prominent, lymphocytes were infrequent, and no mast cells or plasma cells were encountered. After 24 hours, histiocytes appeared with increasing frequency and, later still, fibroblasts and

early fibrosis were seen. In summary, implanted material disappeared rapidly and mild infiltration by neutrophils occurred with the institution of a natural healing process.

Experiment 2.—

Each of 18 guinea pigs received two subcutaneous implantations of insoluble *Ascaris* protein (25 mg.), one wound being sealed and the other left uncovered. Samples of peripheral blood and wound exudate were taken as before and the animals were killed and autopsied at the same intervals.

Blood changes: Fifteen animals showed a transient neutrophilia with mild depression of lymphocytes as in the first experiments, peaks and normal values being recorded at similar times. All but 2 animals showed increased eosinophils (Table II), 8 animals registering abnormal levels; initial values were regained at 24 to 48 hours. Monocytes were inconsistent and no changes in basophils were noted.

TABLE II. EOSINOPHIL LEVELS OF NORMAL UNSENSITIZED GUINEA PIGS GIVEN SUBCUTANEOUS IMPLANTATIONS OF *ASCARIS* PROTEIN, EXPRESSED AS CELLS PER CUBIC MILLIMETER OF BLOOD

	INITIAL	HOURS								
		1	2	3	6	9	12	24	48	72
1	10	400	310	—	—	—	—	—	—	—
2	10	380	560	—	—	—	—	—	—	—
3	120	50	60	—	—	—	—	—	—	—
4	80	100	190	240	—	—	—	—	—	—
5	130	300	210	790	—	—	—	—	—	—
6	0	220	220	330	—	—	—	—	—	—
7	390	—	—	510	770	—	—	—	—	—
8	370	—	—	480	800	—	—	—	—	—
9	210	—	—	450	280	—	—	—	—	—
10	170	—	200	—	240	580	—	—	—	—
11	400	—	420	—	360	660	—	—	—	—
12	230	—	—	—	490	—	210	—	—	—
13	260	—	—	—	390	—	130	—	—	—
14	80	—	—	—	280	—	320	110	—	—
15	0	—	—	—	120	—	300	310	—	—
16	170	—	—	—	180	—	240	70	—	—
17	50	—	—	170	370	—	—	320	110	—
18	80	—	—	290	510	—	—	470	190	—

Legend: The symbol — indicates that no count was made.

Exudates: Smears became cellular at about 3 hours, monocytes and neutrophils being the most frequent leukocytes although lymphocytes were also seen. Some lymphocytes contained basophilic cytoplasmic granules whereas others had a diffuse cytoplasmic basophilia reminiscent of plasma cells. A few monocytes also contained granular cytoplasmic inclusions. As the granulocytic reaction increased, monocytes and lymphocytes became less conspicuous; with few exceptions eosinophils were not seen before 6 hours and were seldom prominent in the smears. At 24 hours, nearly all cells were neutrophils although occasional histiocytes, sometimes with cytoplasmic inclusions, began to appear at this time. No basophils were seen.

Skin lesions: Sealed and open lesions again showed little difference, and each section included a small epidermal wound extending into the upper dermis.

In all sections this wound contained red cells, fibrin, and easily recognized fragments of *Ascaris* protein, including ova. Little cellular reaction was detected before 3 hours, when, in the tissue adjacent to the wound, dilated capillaries were noted with margination and emigration of mature neutrophils, monocytes, and rare eosinophils. At 6 hours, the tissue was loosely infiltrated by leukocytes, mostly neutrophils, and the protein implant invested by a thin rim of similar cells. Later, when the layer of investing leukocytes was much thicker, the implant now bore an irregular and scalloped outline (Fig. 1). At the same time many of the neutrophils contained dark cytoplasmic granules

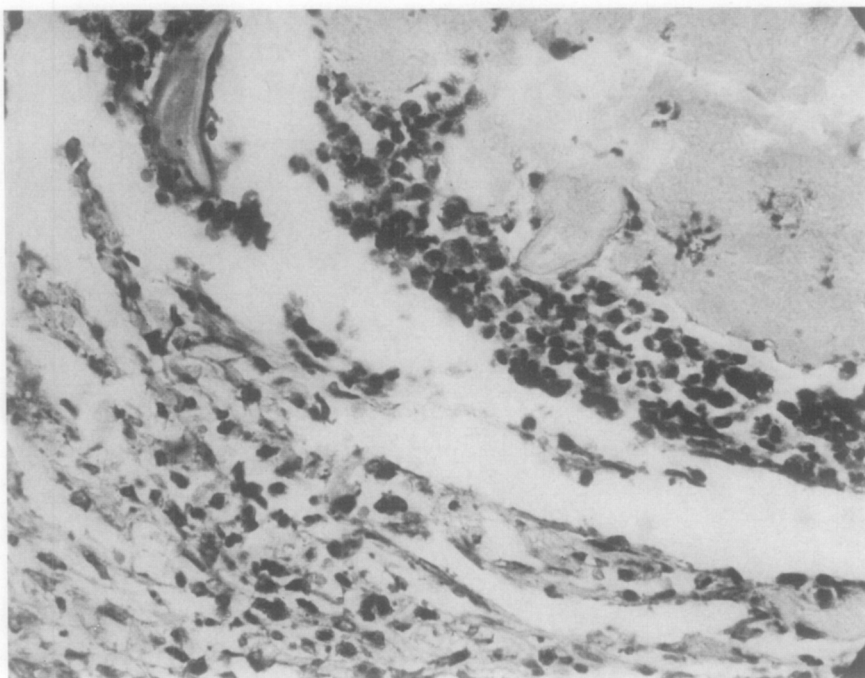


Fig. 1.—*Ascaris* protein implant invested by layers of phagocytic leukocytes and showing an irregular scalloped outline. Cells closest to the implant are mostly neutrophils. Eosinophils are most frequent in the outer edge of this layer and in the loose connective tissue behind. This is from a 12-hour lesion in an unsensitized guinea pig (Exper. 2). (Hematoxylin and eosin, $\times 720$; reduced $\frac{3}{4}$.)

while others were frankly degenerate, frequently associated with large mononuclear phagocytes. Eosinophils began to appear prominent at 12 hours and, while a few lay close to the protein, most were found behind the neutrophils and in advance of the loose accumulation of histiocytes that appeared at about 24 hours. There was no sign of vascular emigration of histiocytes; instead there were many examples of local histiocyte formation in the tissues deep to the wound. Here many capillaries and small arterioles had thickened walls composed of layers of mononuclear cells extending outward, forming a collar. Some outer cells were only tenuously attached to the vascular proliferation, whereas many identical cells lay free in the perivascular tissue. These were indistinguishable from tissue histiocytes (Fig. 2). As active emigration of neutrophil



Fig. 2.—A, proliferation of capillary wall with formation of hystiocytic elements in 48-hour lesion in unsensitized guinea pig (Exper. 2). (Hematoxylin and eosin, $\times 900$; reduced $\frac{3}{4}$.)

B, Proliferation of arteriolar wall and extension of reaction into perivascular tissues forming a collar of mononuclear cells. When lying free in the tissues, these are indistinguishable from tissue histiocytes. A few eosinophils lie in the perineural lymphatics. This is from a 72-hour lesion in a sensitized guinea pig (Exper. 3). (Hematoxylin and eosin, $\times 250$; reduced $\frac{3}{4}$.)

leukocytes slackened pace at about 36 hours, the area of cellular infiltration gradually became more circumscribed and now a familiar pattern emerged. A central area contained *Ascaris* protein remnants and large numbers of neutrophil granulocytes, metamyelocytes, and even myelocytes, many being degenerate and frankly necrotic. This central zone was surrounded by a wide band of histiocytes, some more central cells actively phagocytosing adjacent cellular debris; occasional bi-nucleate forms were seen. Eosinophils were sprinkled throughout the lesion, often close to the histiocytes but no phagocytosis of eosinophils was observed. Eosinophils were also seen in the outer loose connective tissue, frequently lying within dilated lymphatics of the dermal papillae or in the deep perineural lymphatics (Fig. 3). No mast cells were seen. In summary, this lesion showed moderate neutrophil infiltration and partial destruction of the foreign protein with necrosis of the neutrophils; there was accumulation of eosinophils and a well-marked histiocytic response with the eventual formation of a circumscribed foreign body granuloma.

TABLE III. EOSINOPHIL LEVELS IN SENSITIZED GUINEA PIGS FOLLOWING A SECOND SUBCUTANEOUS IMPLANTATION OF ASCARIS PROTEIN, EXPRESSED AS CELLS PER CUBIC MILLIMETER OF BLOOD

	BEFORE SENSI- TIZATION	BEFORE CHAL- LENCE	HOURS								DAYS 6
			1	2	3	6	12	24	48	72	
1	140	180	210	120	300	—	—	—	—	—	—
2	80	70	480	450	310	—	—	—	—	—	—
3	200	110	120	120	240	—	—	—	—	—	—
4	290	160	—	—	300	490	—	—	—	—	—
5	30	120	—	—	40	210	—	—	—	—	—
6	80	90	—	—	110	70	—	—	—	—	—
7	140	120	—	—	210	330	400	—	—	—	—
8	160	80	—	—	60	280	320	—	—	—	—
9	260	190	—	—	290	400	410	—	—	—	—
10	180	100	180	140	290	500	390	200	—	—	—
11	100	90	280	300	300	320	290	390	—	—	—
12	220	100	—	—	—	520	570	410	—	—	—
13	120	100	—	450	420	420	—	330	390	—	—
14	150	80	—	—	—	330	—	410	360	—	—
15	80	40	—	—	—	240	400	180	220	190	—
16	10	120	—	—	—	180	510	160	120	240	—
17	120	20	—	—	—	190	220	440	300	120	170
18	180	100	—	—	330	450	510	660	190	170	190

Legend: The symbol — indicates that no count was made.

Experiment 3.—

Each of 18 guinea pigs received two subcutaneous implantations of insoluble *Ascaris* protein (25 mg.), both wounds being sealed. Six weeks later this operation was repeated and this time one wound was sealed and the other left uncovered. After the first implantation, no blood or exudate studies were undertaken, but after the second implantation, samples of peripheral blood and exudate were taken as before. The animals were killed at 3, 6, 12, 24, 48, and 72 hours and, also, at 6 days, and were autopsied.

Blood changes: After primary implantations no regular blood observations were made, but after the second implantation, an increase in neutrophils and

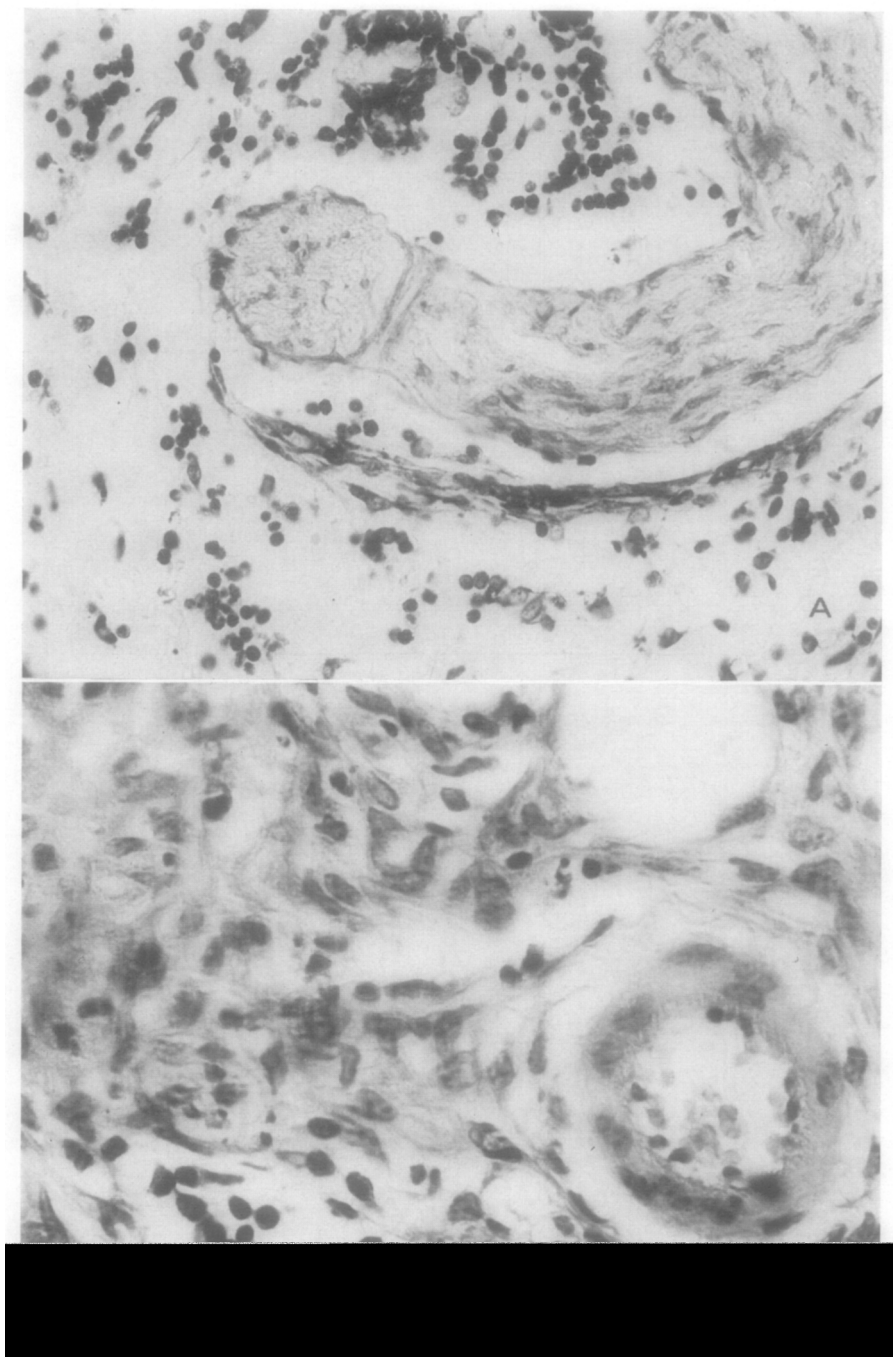


Fig. 3.—A, Accumulation of eosinophil leukocytes within dilated lymphatic channels in 48-hour lesion in unsensitized guinea pig (Exper. 2). There is now little or no emigration of leukocytes from the adjacent blood vessel. (Hematoxylin and eosin, $\times 980$; reduced $\frac{3}{4}$.)
B, Accumulation of eosinophil leukocytes in dilated perineural lymphatics in a 48-hour lesion in sensitized guinea pig (Exper. 3). (Giemsa, $\times 540$; reduced $\frac{3}{4}$.)

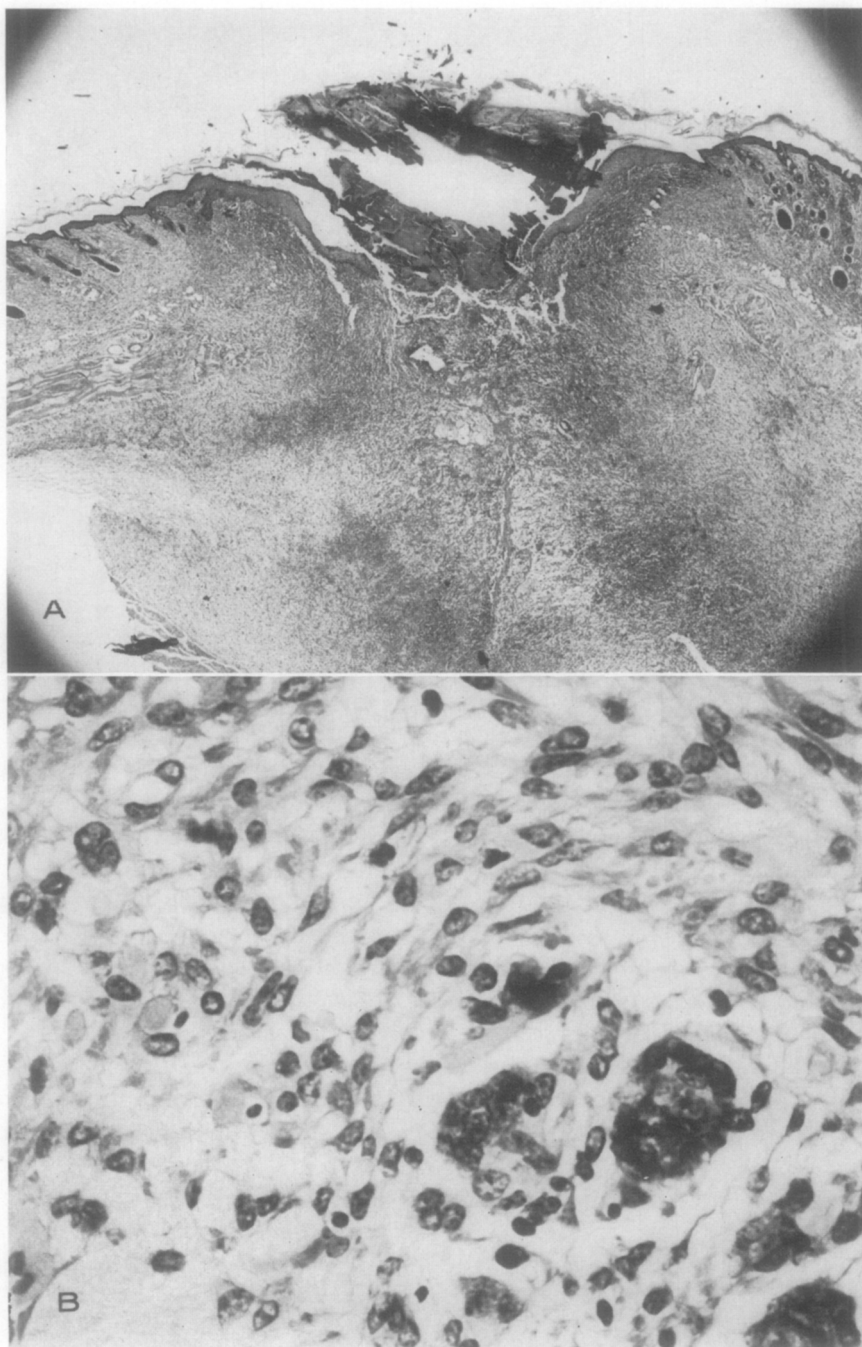


Fig. 4.—A, Fully developed granulomatous lesion at 6 days in sensitized guinea pig. (Exper. 3). (Hematoxylin and eosin, $\times 25$; reduced $\frac{3}{4}$.)

B, Detail of Fig. 4 showing giant cells. (Hematoxylin and eosin, $\times 980$; reduced $\frac{3}{4}$.)

fall in lymphocytes were observed in all but 3 animals. Eosinophils rose above initial levels in 16 animals, reaching abnormal levels in 7, normal levels being regained at 24 to 48 hours. Monocytes behaved irregularly, some transient rises being recorded; basophils showed no changes.

Exudates: In this experiment the course of cytologic events was almost identical to that of Experiment 2. A well-marked nuclear shift in neutrophils and some prominence of eosinophils were noted at 6 hours; monocytes, large and small lymphocytes, basophilic lymphocytes, and histiocytes were observed as before; no basophils were seen.

Skin lesions: Histologically, the lesion developed as in Experiment 2 with only minor differences. The histiocytic response was perhaps more rapid and a zone of epithelioid cells with occasional giant cells (Fig. 4, *A* and *B*) and peripheral fibrosis developed. Eosinophils were again scattered widely throughout the lesion and were observed within lymphatics (Fig. 3, *B*). No mast cells were seen.

DISCUSSION

Following implantation of *Ascaris* materials, the blood of most animals showed a rapid, transient neutrophilia and simultaneous fall in lymphocytes, initial levels being regained by 24 hours. Absent from control animals, these changes may be regarded as a response to the irritant substances, perhaps in the nature of a stress phenomenon as described by Whitby and Britton.¹⁴ Eosinophils responded readily to polysaccharide but less promptly to protein implants. Though more potent in producing blood eosinophilia, the polysaccharide was less effective in producing tissue changes, this perhaps being related to its greater solubility. This solubility probably also accounts for the rapid disappearance of the material from the implantation site, either by absorption or exudation, and explains the poor cellular reaction in the tissues as the polysaccharide could be removed with minimal extravascular leukocytic activity. Under these circumstances, healing would be rapid and uneventful. On the other hand, the sluggish, more uncertain blood eosinophilia after the protein implant may reflect the slower release of chemotactic or eosinotactic substance, this implanted material necessitating preliminary breakdown by readily available biochemically potent elements—leukocytes. Hence the marked tissue infiltration by neutrophils with eosinophils following behind as disintegration of the protein progressed. In the study of these early events one can attempt to reconstruct the sequence of cell movements, and, in the light of accrued experimental knowledge, discuss cellular functions and the purpose of this reaction.

The Neutrophil Leukocyte.—After protein implantation, neutrophils, readily available from the body reserves,¹⁵ increase in the blood stream, and, in the area of injury, sequester and become marginated in the dilated capillaries, as happens where endothelial cells are damaged,^{16, 17} and thence pass into the tissues. In sections of the reaction at 3 hours, extravascular neutrophils, usually

mature segmented forms, lie along tissue planes in the direction of the implant; in more severe or prolonged reactions deeper tissue planes and vessels are also involved. The cells eventually reach the protein which is invested and subjected to enzymatic and phagocytic attack, the edges of the implant soon becoming scalloped and "moth-eaten." At 6 hours or later, early degenerative and necrotic changes appear in the neutrophils, and phagocytosis of nuclear remnants and granules by monocytes and histiocytes becomes increasingly evident. With the stimulus of these necroses the neutrophilic infiltration intensifies and less mature forms, including bands, metamyelocytes, and myelocytes, stream from the dilated vessels around the lesion. The implant is more deeply invaded and ova penetrated and disrupted while, with dark granules and vacuolated cytoplasm, neutrophils continue to degenerate and die. As the reaction subsides, about 36 hours or later, neutrophil emigration from blood vessels diminishes and ceases, these cells now being largely confined to the central inflammatory zone; histiocytic phagocytosis is very active there. Few neutrophils are seen outwith this area and are only infrequently encountered in outer tissue spaces or lymph channels.

The Eosinophil Leukocyte.—Slower to respond than neutrophils, eosinophils increase in the blood stream and pass into the tissues in the area of injury, moving toward the protein. Initially few in numbers, eosinophils slowly accumulate and are prominent in the later stages, when they are scattered throughout the area though more abundant in the outer part of the central zone. As emigration subsides they are, unlike the neutrophil, still seen in outlying areas, frequently within dilated tissue spaces or lymphatics. Sometimes eosinophils and histiocytes lie side by side and, occasionally, such a histiocyte, like others not associated with eosinophils, may contain irregular red cytoplasmic inclusions. Frequently observed,¹⁸ particularly in hyperergic inflammatory states, this has been taken as evidence of histiocytic phagocytosis of the eosinophil.¹⁹ The infrequency and variability of these findings and the morphologic characteristics tend to cast doubt upon this, although electron microscope studies, such as Welsh and Geer²⁰ used for mast cell granules, might be more definite. However interpreted, these were uncommon findings and the progression of eosinophils into the subcutaneous lymphatics would seem the normal sequel, perhaps with eventual re-entry into the blood stream.

The Monocyte.—No regular response of monocytes was observed in the blood stream but in sections, these were perhaps the earliest cells to emigrate from the vessels and to appear in smears of the wound exudate. These early arrivals soon engaged in phagocytic activity, acquiring cytoplasmic inclusions but, as the neutrophil infiltration increased, became less evident and apparently played little part in subsequent events.

The Histiocyte.—This designation is applied to a cell with round or slightly oval nucleus with diffuse fine chromatin and prominent nucleolus. The cytoplasm is abundant with an irregular outline and frequent protrusions,

sometimes long and filamentous like a fibroblast. Of a pale eosinophilic color, the cytoplasm frequently contained cytoplasmic inclusions—coarse and fine, acidophilic and basophilic.

These appeared late in the reaction, most commonly in the area underlying the zone of protein and neutrophils. No emigration of these cells from blood vessels was ever seen; instead they were formed locally by the proliferation of capillaries, these structures being known to retain much of their primitive multipotency, including phagocytosis and the formation of reticuloendothelial cells.^{17, 18, 21} First accumulating in a loose arrangement, the histiocytes later formed a compact zone of cells around the disintegrating protein, some of the more centrally placed cells being phagocytic and occasionally formed giant cells. The deposition of a few strands of fibrous tissue around the periphery of the histiocyte layer completed the formation of the foreign body granuloma.

In unsensitized animals, tissue reactions similar to these have previously been produced by extracts of *Ascaris* and by live intact ova,^{4, 22} but these did not appear for 14 days or more, this delay being held as evidence of an immunologic mechanism. In other *Ascaris* hypersensitivity studies, Speirs and Dreisbach²³ regarded some early eosinophilic responses as nonspecific. In contrast, the lesions studied here developed rapidly and the close similarity of the changes observed in both sensitized and unsensitized animals would seem to discount any significant immunologic influence. This property of being able to bring about changes firmly associated with immunologic mechanisms, such as anaphylactoid shock, eosinophilia, and granulomatous lesions, appears to reside in most derivatives of *Ascaris* tissue, including polysaccharide, polypeptides, and glucolipids⁶ and, as we have seen, insoluble protein. Thus, it seems likely that in some or all of these materials lie the primary toxic factor or factors which initiate the pathologic events following antigen-antibody interaction. There are present in these lesions, changes associated with increased histamine, such as capillary proliferation and phagocytosis¹⁷; there are basophilic lymphocytes, which Tompkins²⁴ regards as evidence of sublethal nuclear damage, and the degeneration and necrosis of great numbers of neutrophil leukocytes, all of which suggest the local accumulation of cytotoxic substances. Also present is the eosinophil, a robust cell which can phagocytose such toxic material as histamine-rich mast cell granules²⁰ and can transport anaphylactogenic material in experimental animals.⁷ It is seen within this unfavorable environment where it penetrates almost to the center of the lesion, persists in the tissue with little evidence of degenerative change, and emerges, apparently undamaged, to enter the lymph channels draining the area. These observations lend strong support to the concept of eosinophil function as an agent, absorbing, neutralizing, removing, or otherwise dealing with toxic substances perhaps lethal to other cells.

SUMMARY

The reaction in the blood and subcutaneous tissues of the guinea pig to implanted *Ascaris* polysaccharide and *Ascaris* protein has been studied.

Soluble polysaccharide produced a brisk eosinophilia of the blood with minimal response in the tissues. The insoluble protein produced erratic minimal changes in the blood eosinophils but stimulated a well-marked tissue reaction of the foreign body granuloma type in which eosinophils were prominent.

The observed sequence of histologic events yields valuable clues to the parts played by the individual cell types and to the essential nature and purpose of the tissue reaction.

The true nature of eosinophil function and its pathologic significance are tentatively discussed.

REFERENCES

1. Speirs, R. S.: Physiologic Approaches to an Understanding of the Function of the Eosinophils and Basophils, *Ann. New York Acad. Sc.* **59**: 706, 1955.
2. Waalkes, T. B., and Coburn, H.: The Role of Platelets and the Release of Serotonin and Histamine During Anaphylaxis in the Rabbit, *J. ALLERGY* **30**: 394, 1959.
3. Machebouf, M., and Mandoul, R.: A propos de la toxicité des extraits d'*ascaris*, *Compt. rend. soc. de biol.* **130**: 1032, 1939.
4. Campbell, D. H.: Experimental Eosinophilia With Keratin From *Ascaris Suum* and Other Sources, *J. Infect. Dis.* **71**: 270, 1942.
5. Rocha y Sylva, M., and Grana, A.: Anaphylaxis-like Reactions Produced by *Ascaris* Extracts, *Arch. Surg.* **52**: 523, 1946.
6. Deschiens, R.: Les substances toxiques vermineuses, leur pouvoir pathogene, leur identification, *Ann. Inst. Pasteur* **75**: 397, 1948.
7. Godlowski, Z. Z.: Transportation of the Anaphylactogenic Property by Eosinophil, *Brit. J. Exper. Path.* **29**: 511, 1948.
8. Sprent, J. F. A.: On Toxic and Allergic Manifestations Caused by Tissues and Fluids of *Ascaris*: Effect of Different Chemical Fractions on Worm-Free, Infected and Sensitized Guinea Pigs, *J. Infect. Dis.* **86**: 146, 1950.
9. Vaughn, J.: Stimulation of the Eosinophil Leucocyte, *J. Path. Bact.* **64**: 91, 1952.
10. Vaughn, J.: On the Function of the Eosinophil Leukocyte, *Blood* **8**: 1, 1953.
11. Kolouch, F., Jr.: Lymphocyte in Acute Inflammation, *Am. J. Path.* **15**: 413, 1939.
12. Rebutck, J. W., Monto, R. W., Monahan, E. A., and Riddle, J. M.: Potentialities of the Lymphocyte With an Additional Reference to Its Dysfunction in Hodgkin's Disease, *Ann. New York Acad. Sc.* **73**: 8, 1958.
13. Barnett, C. W.: The Unavoidable Error in Differential Count of Leukocytes of the Blood, *J. Clin. Invest.* **12**: 77, 1933.
14. Whitby, L. E. H., and Britton, C. J. C.: Disorders of the Blood, ed. 8, New York, 1956, Grune & Stratton, p. 82.
15. Craddock, C. G., Jr., Perry, S., Ventzke, L. E., and Lawrence, J. S.: Evaluation of the Marrow Granulocyte Reserves in Normal and Disease States, *Blood* **15**: 840, 1960.
16. Forbus, W. D.: Reaction to Injury, vol. 1, Baltimore, 1943, The Williams & Wilkins Company, p. 129.
17. Zweifach, B. W.: The Arterial Wall, edited by A. I. Lansing, Baltimore, 1959, The Williams & Wilkins Company, p. 35.
18. Grawitz, P. B.: Der Beginn des Entzündungsprozessen im Bindegewebe, *Virchow's Arch. path. Anat.* **321**: 62, 1951.
19. Speirs, R. S.: Advances in the Knowledge of the Eosinophil in Relation to Antibody Formation, *Ann. New York Acad. Sc.* **73**: 283, 1958.
20. Welsh, R. A., and Geer, J. C.: Phagocytosis of Mast-Cell Granule by the Eosinophil Leukocyte in the Rat, *Am. J. Path.* **35**: 103, 1959.
21. Zweifach, B. W.: An Analysis of the Inflammatory Reaction Through the Response of the Terminal Vascular Bed to Micro-Trauma, *Rev. Canad. Biol.* **12**: 79, 1953.
22. Chowdhury, A. B., Kean, B. H., and Browne, H. G.: Inoculation of Helminth Eggs Into Animals Eyes, *Am. J. Path.* **36**: 725, 1960.
23. Speirs, R. S., and Dreisbach, M. E.: Qualitative Studies of Cell Responses to Antigenic Injections in Normal Mice, *Blood* **11**: 44, 1956.
24. Tompkins, E. H.: The Conversion of Lymphocytes With "Atypical Nuclei" Into Plasma Cells of the Marschalkó Type, *Am. J. Path.* **37**: 175, 1960.