The Function of the Eosinophile Leukocyte

By JOHN VAUGHN, M.D.

SINCE 1846 when Wharton Jones discovered "the coarse granular corpuscle," later designated by Ehrlich as "the eosinophile leukocyte," this blood cell has occupied the attention of many workers in the field of hematology. In the early part of this century, bitter controversies raged about the origin of the eosinophile leukocyte, Ehrlich and Wiedenreich being among the principal contenders. The various points at issue and their final resolution have been fully discussed by Ringoen.

The bright red granules have occasioned a great deal of speculation about their nature and over the years have been alleged to contain, or to consist of phosphorus, iron, glycogen, protein and tyrosine, while more recently the presence of lipoid has been demonstrated in them. At present, the general opinion would seem to be that the granules contain a phospholipid substance with probably a protein center and desoxyribonucleic acid.

The function of the cell has naturally received an equally full measure of attention, and one of the earliest theories was put forward by Heidenhain in 1888. He noticed that the intestinal mucosa of starving animals contained fewer eosinophils than did the mucosa of normal animals, and deduced therefrom that these cells played some part in nutrition, a view subsequently upheld by the observations of others.

Later, Rous made differential leukocyte counts on the lymph of dogs on various diets and while his findings were in keeping with the ideas of Heidenhain, at the same time he made the interesting observation that the proportion of eosinophils in the lymph rose higher after a meal which was rich in protein than after one rich in carbohydrate. This association of the eosinophil and protein has since been frequently stressed, especially with regard to anaphylactic and related responses.

In 1895, Mesnil demonstrated the phagocytic properties and the chemotactic nature of the eosinophile leukocyte, findings which have been amply confirmed. Later, Chauffard and Boidin, studying the eosinophil reaction surrounding hydatid cysts, suggested that the eosinophils neutralized small quantities of fluid which leaked through the cyst wall, and Weinberg and Séguin demonstrated how the eosinophile leukocyte could in fact neutralize the toxic properties of small amounts of hydatid fluid in vitro. It is now generally conceded that the eosinophil plays some part in dealing with toxic material, this material probably being derived from protein. The association of the eosinophile leukocyte with allergic states and anaphylaxis has long been recognized.

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Recently in this field an interesting experiment was carried out by Godlowski\textsuperscript{27} who produced a peritoneal exudate in guinea pigs by repeated injections of antigenic material. Since the exudate was rich in eosinophils, he discovered that an extract could bring about an anaphylactic response in the uterus of a guinea pig which had already been sensitized to the same original antigenic substance. As this effect was not observed when the uterus had been sensitized to a different antigen, or when the exudate was poor in eosinophils, he concluded that the eosinophil carried some anaphylactogenic property. The complexity of the antigen-antibody reaction led Campbell\textsuperscript{28} to attempt to dissociate eosinophilia and allergic states. To this end he used an insoluble fraction of ascaris whole worm to produce an eosinophilia in guinea pigs by a single injection. As the insoluble material remained in the tissues for several days, however, the possibility of an antigen-antibody reaction having taken place could not be excluded.

Ascaris extracts have been used on many occasions in experimental work on animals\textsuperscript{29-31} but primarily in the investigation of the problems of allergy and shock. In those accounts that have recorded changes in the eosinophils, these have occurred only after repeated injections of ascaris extracts, thus probably accompanying anaphylactoid or other allergic responses. Recently the author\textsuperscript{32} reported a series of experiments in which soluble protein-free extracts of ascaris were used to produce a transient eosinophilia in guinea pigs following the use of a single injection. On this occasion great care was taken to ensure that the animals had not been exposed to any antigenic materials and most were actually bred for the experiments. Thus it was hoped to exclude the possibility of antigen-antibody reactions taking place during the tests. From the findings, evidence was adduced which suggested that stimulation of the eosinophile leukocyte is most probably a direct chemical action upon the bone marrow either by histamine or by a substance closely related to it.

In the present investigation, the histologic changes, particularly the distribution of eosinophils accompanying the eosinophilia produced by injection of ascaris extract, have been studied and compared with changes of histamine-induced eosinophilia, and those of eosinophilia as it occurs naturally in the guinea pig. It was hoped thereby to achieve a fuller comprehension of the eosinophilic state, and perhaps at the same time gain some idea of the path of the eosinophile leukocyte in the body and the mystery of its function.

MATERIALS

\textit{Ascaris Extract}

An extract of whole \textit{Ascaris suum} was made by a modification of the method of Macheboul and Mandoul,\textsuperscript{29} the exact details being reported elsewhere.\textsuperscript{32}

The whole worm material, after being pulped, is precipitated by trichloracetic acid and the supernatant protein-free fluid dialysed against normal saline. From this fluid a white substance is precipitated by alcohol and after re-solution in water and re-precipitation several times, it is dried first in alcohol and then in acetone. Chemically this material is a polysaccharide of high molecular weight. It possesses the property of stimulating the production of eosinophile leukocytes in guinea pigs when injected in saline solution.

\textit{Histamine Acid Phosphate}

A standard pharmaceutical preparation in tablet form was used, the tablets being dissolved in saline immediately prior to injection. The doses given are expressed as equivalent weights of histamine.
ANIMALS

In investigations of this nature, rabbits and guinea pigs were the animals most commonly used. The latter proved more convenient to handle and were more readily available. It has been observed\(^{28}\) that some older guinea pigs tend naturally to develop an eosinophilia; this has sometimes been attributed to intestinal parasites such as the helminths, *Paraspidodera uncinata*, *Obeliscoides cariculi*, etc.\(^{29}\) Consequently, young guinea pigs were selected in order to avoid using parasitised animals as far as possible. Most of the guinea pigs used were less than 300 grams in weight. To obviate the possibility of obscure allergic responses affecting the experiments, guinea pigs which had already been used for any other purpose whatsoever were rejected.

Scarborough\(^{34}\) quotes the normal average eosinophil levels in the blood of guinea pigs as 500 per cu. mm. In these experiments, however, all the animals—unless otherwise deliberately chosen—had eosinophil counts much below this level and indeed many had initial counts of 100 eosinophils or less per cu. mm. of blood.

Injections were given intraperitoneally; a simple and easily standardized procedure. All animals were killed by chloroform.

METHODS

**Eosinophil Counts**

The direct eosinophil count is more expedient and probably more accurate than the time-consuming differential leukocyte count. A nick was made in the ear of the guinea pig and after discarding the first drops,\(^{35}\) blood was withdrawn into a white cell pipet and diluted with Dungar's fluid,\(^{36}\) after which the pipet was shaken for 30 seconds only, as recommended by Thorn, et al.\(^{37}\) Two hemocytometer chambers were filled from each pipet and counts made, the number of eosinophils being expressed to the nearest 10 per cu. mm.

**Histologic Preparations**

At autopsy, which was performed immediately after death, specimens of the following tissues were removed in each case: lung; hilar tissue, which included bronchus, pulmonary vessels and the hilar lymph node; small intestine; spleen and parietal peritoneum. These were fixed in Bouin's fluid or formol-saline, paraffin sections prepared, and stained by hematoxylin-eosin.

**Observation of the Normal**

In order that tissue changes following the injection of toxic materials into guinea pigs might be more clearly evaluated, normal tissue was obtained from 5 young guinea pigs. These animals had their blood eosinophil levels estimated on two occasions 24 hours apart. Immediately after the second estimation they were killed, material being removed for histologic study at autopsy.

**Results**

Table 1 shows the eosinophil levels observed in the blood of 5 normal guinea pigs.

<table>
<thead>
<tr>
<th>Guinea pig No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours before death</td>
<td>140</td>
<td>70</td>
<td>80</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>At death</td>
<td>40</td>
<td>130</td>
<td>40</td>
<td>130</td>
<td>20</td>
</tr>
</tbody>
</table>

The figures expressed represent the number of eosinophile leukocytes per cu. mm. blood.

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\(^{28}\) Jonas.\(^{29}\)\(^{30}\)\(^{31}\)\(^{32}\)\(^{33}\)\(^{34}\)\(^{35}\)\(^{36}\)\(^{37}\)
FUNCTION OF EOSINOPIHLE LEUKOCYTE

Histology

The peritoneum was normal and no eosinophils were seen. In some of the lungs there were infrequent patches in which the walls of the alveoli were thickened. These areas, which were quite haphazard in distribution, contained alveolar epithelial cells, septal cells, macrophages and a few lymphocytes, the picture thus being mononuclear. The alveolar capillaries were normal and no granular leukocytes were seen.

The larger blood vessels and bronchi appeared normal and no eosinophile leukocytes were present in the perivascular or peribronchial tissues, although in one section an occasional eosinophil was seen within the lumen of a bronchus. The lymphatic channels, the hilar node, main bronchus and vessels were normal.

In the intestine very small numbers of eosinophils were seen in the mucosa and submucosa in three of the sections. No eosinophils were seen in sections of spleen.

Experimental Eosinophilia I

Twenty normal unsensitized guinea pigs were selected and after estimating their blood eosinophil levels, they were given injections of 30 mg. ascaris extract, dissolved in sterile saline. The animals were thereafter killed in groups of 4 at 1 hour, 3 hours, 6 hours, 12 hours and 24 hours after injection. Eosinophil counts were made where possible at 6, 12 and 24 hours and in all cases immediately before death. At autopsy, tissues were removed for histologic study.

Results

Table 2 shows the eosinophil levels observed in the blood of 20 normal guinea pigs injected with ascaris extract.

Table 3, albeit brief and to some extent arbitrary, gives a ready indication of the distribution of eosinophils which was found in the guinea pig tissues. Supplementary notes are given where it was felt that a fuller description was necessary. No macroscopic abnormalities were noted at autopsy.

Histology

At 1 hour. In each lung there were dense peribronchial areas in which there was thickening of the alveolar walls. These patches were not seen in other than peribronchial distribution. In the thickenings were observed dilatation and engorgement of the alveolar capillaries with swelling of the alveolar epithelium. No granular leukocytes were seen.

In two sections some of the larger vessels contained intravascular eosinophils which were arranged along the endothelium in “pavement” fashion as if prior to emigration, while scanty eosinophils were noted in the surrounding connective tissue. The bronchi, parabronchial lymph nodules and hilar node were normal. In the main bronchus, eosinophils in small numbers were seen in the submucosa.

Eosinophils were present in the submucosa of the intestine, near the small vessels and also in the submucosa.

At 3 hours. The areas of thickening of the alveolar walls were now much more widespread though denser nearer the bronchi and vessels. In addition to the
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alveolar changes already noted there were a few scattered eosinophils in the walls.

Pavementation of eosinophils within the blood vessels was a common occurrence, and moderately large numbers of these cells were present in the perivascular and peribronchial connective tissue. A few had invaded the bronchial mucosa, an occasional cell lying free in the lumen.

Lymphatics and hilar node were normal but the main bronchus had many eosinophils in the mucosa and submucosa.

**Table 2—Eosinophil Levels Observed in the Blood of Normal Guinea Pigs after Injection with Ascaris Extract**

<table>
<thead>
<tr>
<th>No.</th>
<th>Killed at</th>
<th>Initial</th>
<th>Time after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr.</td>
</tr>
<tr>
<td>1</td>
<td>1 hour</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>1 hour</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>1 hour</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1 hour</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>3 hours</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>3 hours</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>3 hours</td>
<td>50</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>3 hours</td>
<td>70</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>6 hours</td>
<td>70</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>6 hours</td>
<td>40</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>6 hours</td>
<td>60</td>
<td>—</td>
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<tr>
<td>12</td>
<td>6 hours</td>
<td>100</td>
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<tr>
<td>13</td>
<td>12 hours</td>
<td>100</td>
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<td>14</td>
<td>12 hours</td>
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<td>15</td>
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<td>16</td>
<td>12 hours</td>
<td>110</td>
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<tr>
<td>17</td>
<td>24 hours</td>
<td>110</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>24 hours</td>
<td>40</td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>24 hours</td>
<td>90</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>24 hours</td>
<td>70</td>
<td>—</td>
</tr>
</tbody>
</table>

The figures expressed represent the number of eosinophile leukocytes per cu. mm. blood.

In the intestine eosinophils were seen pavemented within the small vessels of the submucosa and many others lay in the connective tissue between the vessels and the mucosal epithelium, some arranged in tiny "processions."

At 6 hours. Areas of thickening of the alveolar walls were now more extensive, in some specimens involving the whole section and amounting in places to complete consolidation. By far the greater number of eosinophils was observed in the broncho-vascular connective tissue but some had invaded the bronchial mucosa, while others were dotted along the alveolar walls.

A few eosinophils were seen in one of the hilar nodes.

At 12 hours. Widespread thickened areas with patchy consolidation was a common finding and in addition to the usual eosinophilic infiltration of the bronchi and surrounding tissue, an occasional cell was seen in the parabronchial lymph nodules and in the hilar nodes.
| Killed at | 1 hour | 2 hours | 3 hours | 4 hours | 5 hours | 6 hours | 7 hours | 8 hours | 9 hours | 10 hours | 11 hours | 12 hours | 13 hours | 14 hours | 15 hours | 16 hours | 17 hours | 18 hours | 19 hours | 20 hours |
|----------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Guinea pig Nos. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Peritoneum | | | | | | | | | | | | | | | | | | | | | |
| Lung | | | | | | | | | | | | | | | | | | | | | |
| alveolar walls | - | - | + | - + | - + | + | - | + | - | ± | - | - | + | - | - | - | + | ± | ± | ± | ± |
| perivascular | - | ± | ± | - | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| peribronchial | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| bronchial mucosa | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| bronchial lumen | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| peribronchial lymph nodules | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Hilar tissue | | | | | | | | | | | | | | | | | | | | | |
| lymph node | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| bronchus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| vessels | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Small intestine | | | | | | | | | | | | | | | | | | | | | |
| mucosa | - | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| submucosa | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| muscle layer | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| peritoneum | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Spleen | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**Key:** ± = scantly numbers of eosinophils seen.  
+ = small numbers of eosinophils seen.  
+++ = moderate numbers of eosinophils seen.  
++++ = large numbers of eosinophils seen.
At 24 hours. The thickened areas were not confined to a peribronchial distribution in most of the sections, eosinophils being prominent. They were also frequently seen in the lymphatic tissue including the hilar node, and in the intestine they were still abundant.

Conclusion

Following an injection of ascaris extract, there appears in the blood of the guinea pig an increased number of eosinophile leukocytes. Early changes take place in the lung, in which the eosinophile leukocyte plays a prominent part. Dilatation of the alveolar capillaries takes place with swelling of the alveolar epithelium with consequent thickening of the alveolar walls. At first these changes occur only in close relation to the large vessels and bronchi but later spread to involve large areas of the lung. These early changes are accompanied or followed by emigration of eosinophile leukocytes from the blood stream into the perivascular connective tissue, whence many eosinophils appear to make their way to the bronchi into whose walls and mucosa they penetrate, some of them reaching the bronchial lumen. Other eosinophils are found in the alveolar walls in comparatively small numbers.

As more and more eosinophils congregate in the lung, some appear within the lymphatic system and the number of cells so observed increases as the migrations of the eosinophils in the lung continue. Later, eosinophils accumulate in the pulp of the spleen.

Experimental Eosinophilia II

Sixteen normal unsensitized guinea pigs were selected and after estimating their blood eosinophil levels they were given injections of 0.25 mg. histamine, dissolved in sterile saline. The animals were thereafter killed in groups of 4 at 1 hour, 6 hours, 12 hours and 24 hours after injection. Eosinophil counts were made where possible at 6, 12 and 24 hours and in all cases immediately before death. At autopsy tissues were removed for histological study.

Results

The changes observed in blood eosinophil levels are shown in table 4. The distribution of eosinophils found in the tissues examined is set out briefly in table 5, supplementary notes being added where further description was thought necessary. No macroscopic abnormalities were noted at autopsy.

Histology

At 1 hour. Peribronchial thickened areas were again seen in the lung and emigrated leukocytes were plentiful, on this occasion a small proportion being neutrophils. Eosinophils could be seen pavemented within the blood vessels, in the peribronchial connective tissue and within the bronchial lumen.

In the intestine small numbers of eosinophils and a few neutrophils were observed mainly in close relation to the blood vessels.

At 6 hours. In the lungs widespread thickened areas dotted with eosinophils were found. Moderate numbers of emigrated cells lay in the broncho-vascular
<table>
<thead>
<tr>
<th>Guinea pig Nos.</th>
<th>1 hour</th>
<th>6 hours</th>
<th>12 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alveolar walls</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>perivascular</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>bronchial</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>bronchial lumen</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>peribronchial lymph nodes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hilar tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lymph node</td>
<td>-</td>
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</tr>
<tr>
<td>bronchus</td>
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<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>+</td>
</tr>
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<td>muscle layer</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>peritoneum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td>-</td>
<td>-</td>
<td>±</td>
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</tr>
</tbody>
</table>

**Key:**
- ± = scanty numbers of eosinophils seen.
- + = small numbers of eosinophils seen.
- ++ = moderate numbers of eosinophils seen.
- +++ = large numbers of eosinophils seen.
connective tissue, the mucosa of the bronchus and in its lumen. Occasionally they were seen in the parabronchial lymphatics and in the hilar node. The submucosa of the main bronchus in two sections showed abundant eosinophils.

At 12 hours. The lung changes, though less marked, were essentially the same as before. Eosinophils were plentiful in the intestine.

At 24 hours. In the lungs, areas of thickening were not so extensive and there was less evidence of emigration of leukocytes. Small numbers of eosinophils were found in the alveolar walls and in the lymphatic tissue.

Conclusion

The injection of histamine into guinea pigs gives rise to an increase in the number of eosinophile leukocytes in the blood.

**Table 4—Eosinophil Levels Observed in the Blood of Normal Guinea Pigs after Injection with Histamine**

<table>
<thead>
<tr>
<th>No.</th>
<th>Killed at</th>
<th>Initial</th>
<th>1 hr.</th>
<th>Time after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 hrs.</td>
</tr>
<tr>
<td>1</td>
<td>1 hour</td>
<td>120</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 hour</td>
<td>30</td>
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<td></td>
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<tr>
<td>3</td>
<td>1 hour</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 hour</td>
<td>130</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6 hours</td>
<td>150</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>6 hours</td>
<td>210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6 hours</td>
<td>20</td>
<td></td>
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<tr>
<td>8</td>
<td>6 hours</td>
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<tr>
<td>9</td>
<td>12 hours</td>
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<td>10</td>
<td>12 hours</td>
<td>130</td>
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<td></td>
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<tr>
<td>11</td>
<td>12 hours</td>
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<td>12</td>
<td>12 hours</td>
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<tr>
<td>13</td>
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<td></td>
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<tr>
<td>16</td>
<td>24 hours</td>
<td>110</td>
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</tbody>
</table>

The figures expressed represent the number of eosinophile leukocytes per cu. mm. blood.

Soon after the injection, changes take place in the lung which are very similar to those which occur after an injection of ascaris extract. The whole course of events in the 2 cases differ possibly in only two ways. Firstly, the histamine response appears if anything to take place more quickly than that of the ascaris extract. Secondly, the histamine response involves some measure of neutrophil as well as an eosinophilic reaction. In the leukocytic migrations in the lung, the penetration of the bronchial mucosa was primarily a feature of the eosinophil, the neutrophil seeming to prefer to settle in the walls of the alveoli. As before, eosinophile leukocytes accumulated in the lymphatic system and spleen in the later stages.

**Natural Eosinophilia**

It was remarked above that some older guinea pigs develop a spontaneous eosinophilia; due to this the choice of animals in previous experiments has been
FUNCTION OF EOSINOPHILE LEUKOCYTE

restricted. In experiments described here, 5 guinea pigs were selected because they had developed such an eosinophilia. Eosinophil counts were made on these guinea pigs 24 hours apart. Immediately after the second count they were killed, autopsies made and histologic material removed for study.

Results

Table 6 shows the eosinophil levels observed in the blood of the 5 guinea pigs studied. No macroscopic abnormalities were noted at autopsy.

Histology

No granular leukocytes were seen in the peritoneum.

All the lungs examined showed areas of thickening of the alveolar walls with diminution of air spaces and in 1 animal (No. 4) the lung section showed almost complete consolidation. These patches consisted of dilated capillaries, alveolar epithelium, macrophages and numerous leukocytes which were mainly eosinophils, with a few neutrophils. Pavementation of intravascular eosinophils, emigration of eosinophils into the broncho-vascular connective tissue, and invasion of the bronchi and penetration of their lumena, were all observed in every section. The parabronchial lymph nodules contained a few eosinophils.

TABLE 6—Eosinophil Levels Observed in the Blood of 5 Selected Guinea Pigs

<table>
<thead>
<tr>
<th>Guinea Pig No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours before death</td>
<td>2662</td>
<td>1320</td>
<td>1390</td>
<td>1580</td>
<td>1820</td>
</tr>
<tr>
<td>At death</td>
<td>2370</td>
<td>750</td>
<td>2180</td>
<td>1350</td>
<td>1080</td>
</tr>
</tbody>
</table>

The figures expressed represent the number of eosinophile leukocytes per cu. mm. blood.

In the hilar tissue the mucosa of the bronchus contained abundant eosinophils while small numbers of them could be seen in the node.

The intestine of 1 of the animals showed a mixed neutrophil-eosinophil reaction in the submucosa but in the remainder of sections relatively few eosinophils were seen.

Large numbers of eosinophils were found in the splenic pulp.

Conclusion

In these 5 guinea pigs, where a natural eosinophilia of some standing was in progress, essentially the same histologic features were observed as in the experimental cases, differing only in so far as greater numbers of eosinophils were seen in the natural cases.

Discussion

Histologically the striking feature of eosinophilia of the blood in guinea pigs is the presence of large numbers of eosinophile leukocytes in the lungs and intestine. The infiltration of these tissues by the eosinophil takes place in a well defined and apparently purposeful way. With great regularity these cells appear in the sub-mucosa and mucosa of the intestine, while in the lungs a definite progression of
the cells from the blood vessel to the bronchus is a constant feature. Thereafter, in the lungs at any rate, the eosinophil is seen lying free within the lumen of the bronchus, or caught up in the lymph follicles, perhaps subsequently to re-enter the bloodstream. In the later stages, eosinophils accumulate in the spleen. This may be a proportional accumulation of circulating eosinophils or on the other hand a selective segregation of effete leukocytes. In the intestine, however, neither extrusion of the eosinophil into the lumen, nor its entry into the lymphatic system was observed though if such were its fate, the eosinophil would have thus described a course similar to that which it describes in the lung. As these two events, namely, discharge into the natural passages, and deposition within the elements of the reticulo-endothelial system, are recognized steps in the process by which the body normally rids itself of spent blood cells, it would seem that at this stage, the eosinophil has accomplished its function.

Several workers, commenting upon the early disappearance of leukocytes from transfused blood have attributed this effect to a selective action on the part of the lung. Bierman et al. have demonstrated by cardiac catheterization and leukocyte counts that an injection of histamine can cause retention within the lung of leukocytes for a period of up to 3 minutes. This is presumably an intravascular retention in view of its transience. Osogoe and Osogoe and Omura, when injecting hemopoietic elements into the bloodstream of rabbits, have observed transitory accumulations of lymphocytes in the small blood vessels of the lung, and on another occasion, blockage of the lung capillaries by myelocytes and megakaryocytes, the smaller cells having no doubt successfully negotiated the intricate narrow of the alveolar capillaries. These events, however, savor of the fortuitous and differ from the purposeful movement of the eosinophils into the lungs of the eosinophilic guinea pigs of the present study.

In a recent review Code suggests that the function of the leukocyte—particularly the eosinophil leukocyte—is to carry histamine. Since there is greater concentration of histamine in normal bone marrow than in normal blood he concludes that all the histamine that a leukocyte carries is acquired before it leaves the marrow. It has already been suggested that the stimulus of the formation of the eosinophil is the direct action of histaminic or similar substance upon the marrow. This, together with the facts that eosinophils are rich in histamine and that Godlowski has demonstrated the ability of the eosinophil to carry anaphylactogenic material, lends considerable support to Code's view. The removal of toxic material however effected, should logically be carried out to some part of the body where such a substance can be dealt with safely. Best and McHenry have shown that the histamine which is normally produced in the body is inactivated by an enzyme, histaminase. Although it is present in many parts of the body, histaminase is mainly found in the small intestine. Thus, the theory of the eosinophil being a carrier of histamine allows us to explain the presence of so many eosinophils in the submucosa of the small intestine shortly after an injection of histamine or other toxic substance (ascaris extract).

The presence of a nonhistaminic anaphylactogenic substance has been demonstrated by Campbell and Nicoll in the lung of a sensitized guinea pig, i.e., one which has been injected with an antigenic substance. The concentration of toxic material found in the lung of that animal, and the presence of large numbers of
functional of eosinophile leukocyte

eosinophils in the lungs of guinea pigs studied here may have some common factor and so the theory of the eosinophil as a carrier of histamine (or other toxic substance) may offer an explanation once again. Against this may be set the fact that no large quantities of histaminase have been reported in the lung but perhaps in an emergency, when the intestinal histaminase is overloaded, some measure of inactivation of histamine may be undertaken by other enzymes.

Thus the existence of an eosinophilia of the blood may indicate that somewhere in the body, histamine or some similar toxic substance is being set free in abnormal quantities, and should these quantities be excessive, then changes are taking place in the lungs.

It is commonly accepted that histamine is released in the course of antigen-antibody reactions, the sequence of events being fundamentally proteolysis by intracellular trypsins. This, together with the fact that some degree of breakdown of normal, abnormal or foreign protein in the tissues is part of many pathologic processes, allows the release of histamine to be postulated in most of the clinical conditions associated with eosinophilia in man, e.g. post-febrile states, helminthic infestations, cancer, therapeutic reactions, allergic states, etc. Even familial eosinophilia may have as its basis some inherent error of metabolism.

The importance of events in the lungs, when with the help of the eosinophil, the guinea pig deals with a toxic material like ascaris extract or histamine would lead one to anticipate that in any similar intoxication in man the eosinophilia of the blood might be accompanied by pulmonary changes. Löffler's original description of pulmonary infiltration associated with an eosinophilia of the blood was soon followed by the recognition of this syndrome in many diseases and in many parts of the world. In China, Engel reported cases of "privet cough," a seasonal allergic affection which although not diagnosed as such, was almost certainly a pulmonary eosinophilic infiltration. Eosinophilic infiltration of the lungs has been diagnosed in asthmatic children, in tropical respiratory disease, and in many parasitic infestations including ascariasis, trichinaisiasis, ancylostomiasis, fascioliasis, strongyloidiasis, schistosomiasis and amoebiasis as well as in a variety of allergic states including periarteritis nodosa.

Viswanathan reported the postmortem examination of a case of tropical eosinophilia and found in the sections of lung that the infiltration seemed to show a peribronchial distribution. This led him to believe that the bronchus was the route by which the toxic material responsible for the reaction had entered the body. A similar appearance was seen in some sections of lung examined in this study where, as the lung changes were subsiding, the patches of alveolar thickenings, dotted with eosinophils, were confined to those areas close to the large bronchi and blood vessels.

**General Conclusion**

The course of an eosinophilia of the blood in guinea pigs involves changes in the lungs and in the intestine in which these structures become infiltrated with eosinophile leukocytes. Close observation of these changes has permitted the tracing of a path which the eosinophil probably follows in its journey through the body. Formed in the bone marrow, the eosinophile leukocyte travels in the blood stream to the lungs or intestine, where leaving the blood vessel it makes its
way through the tissues to the mucosa of the bronchus or intestine. At this point, in the lung at any rate, it either passes into the lumen of the bronchus and is so eliminated from the body, or it is caught up in the lymphatic system whereby it may re-enter the blood stream from which it is extracted and subsequently destroyed by the spleen.

In the light of previous published work, and of the present findings, it is highly probable that the function of the eosinophile leukocyte is to carry histamine, or a histamine-like toxic material from the bone marrow to the tissues for inactivation. This concept of the function of the eosinophil provides a common factor among the widely varied clinical conditions with which eosinophilia of the blood is associated in man, and offers an explanation of the clinical syndrome, eosinophilic infiltration of the lungs.

**SUMMARY**

1. Eosinophilia has been produced experimentally in guinea pigs by the injection of extracts of *Ascaris suum* and histamine, the accompanying histologic changes, particularly the distribution of eosinophils, being noted.

2. The tissues of guinea pigs suffering from a natural eosinophilia have been studied and compared with those of experimental eosinophilia.

3. From these findings a path has been traced which, it is suggested, the eosinophil follows in its course through the body.

4. The function of the eosinophil has been discussed and the hypothesis advanced that its function is the carriage of histamine or a histamine-like toxic material for inactivation.

5. This viewpoint has been considered in relation to the occurrence in man of eosinophilia including eosinophilic infiltration of the lungs.

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