Effect of Horse Serum, Adrenal Hormones, and Histamine on the Number of Eosinophils in the Blood and Peritoneal Fluid of Mice

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IT IS NOW reasonably well established that an increase in the hormonal output of the adrenal cortex leads to a reduction in the number of circulating eosinophils and under certain conditions this is a quantitative relationship. The eosinopenic response has been incorporated into diagnostic tests for pituitary and adrenal activity as well as into assay procedures for cortisone and ACTH. The mechanism producing this eosinopenia is unknown; however, recently a number of workers have suggested a direct action of adrenal hormones producing a destruction of the eosinophils. Other workers were unable to obtain similar results.

Rebuck in a review of the white blood cells, pointed out that "These cells are found in the blood only in passing, and they exert many of their more important functions after leaving the blood." Therefore, it seemed desirable to develop techniques which could be used to study eosinophils simultaneously in blood and other tissues. In this manner, it could be determined whether an eosinopenia of the blood was preceded or followed by a general tissue eosinopenia or eosinophilia. For many years, it has been known that repeated injections of foreign protein will produce a local eosinophilia which may also reflect in the blood stream. If quantitative determinations of the local concentrations of eosinophils could be made, then the direct action of various materials on these cells could be investigated. This paper is a report on the method for determination of eosinophil numbers in the peritoneal fluid of mice, and on the changes in the numbers of these cells in the blood and peritoneal fluid following injections of foreign protein, cortisone, compound F, epinephrine and histamine. Attempts were also made to determine if these materials had a direct lytic action on the eosinophils.

MATERIALS AND METHODS

The technique for counting eosinophils using the acetone-phloxine diluent for blood was found to be equally satisfactory for eosinophils in fluid obtained from the peritoneal cavity. The cells appear to be morphologically identical to those found in the blood.

The animals used in these experiments were BBF mice which are the first generation offspring of a cross between C57BL/6 and C57BR/cd strains. All animals used were normal adult males weighing approximately 30 Gm. The mice were isolated in individual cages maintained at 27°C for at least three days prior to the eosinophil determinations. Before the counts were taken the animals were placed in a 4 liter jar and warmed under a 100 watt lamp. After 3 to 5 minutes the mice became active; they were then held in cheese cloth and their tails gently washed in warm water. The tail was dried.

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and a diagonal cut was made through either the ventral or one of the lateral tail veins. The first drops of blood were wiped away with a soft cleansing tissue and succeeding drops were drawn into a WBC pipet for the eosinophil determination. The mice then received a 1 ml. intraperitoneal injection of 0.9 per cent saline to increase the total volume of peritoneal fluid. Three to 5 minutes later they were anesthetized with ether, placed on their backs, and taking care to avoid hemorrhage, the peritoneal cavity was opened. Four samples of fluid were taken systematically from the four quadrants of the peritoneal cavity. The fluid was drawn into standard WBC pipets and diluted 1:20 with the phloxine-acetone diluent. One chamber of an eosinophil counting slide was loaded from each of four pipets, and the number of eosinophils counted.

It was found that the above technic for obtaining blood and peritoneal fluid for eosinophil determinations could be performed very rapidly and simply without sacrificing the animals. The average time for taking both counts was 5 minutes per animal when they were handled in groups of 6 or more. Each animal was used only once in any experiment; however, it would have been possible to reuse it on other experiments if desired. In later experiments, it was found that the eosinophil levels throughout the peritoneal cavity were consistent, and only two samples of fluid were needed for a sufficiently accurate determination. This confirms a similar report by Hilliard and Nash in which they note a relatively even distribution of cells throughout the fluid of the peritoneal cavity.

The standard error of the technic obtained by comparing four pipets of peritoneal fluid from each of 15 animals was two times the square root of the number of cells counted. This was only slightly higher than that obtained for the blood counts.

All the cell counts are given as eosinophils per cu. mm. of blood or diluted peritoneal fluid. The multiplying factor used was 5 for the blood counts and either 5 or 2.5 for the peritoneal counts. However, some of the animals (the ones receiving multiple injections of protein) had peritoneal eosinophil levels too high to be conveniently counted by this method. In such instances a minimum of 400 cells for each sample were counted and the multiplying factor determined.

The cortisone was obtained from Merck & Co., Inc. It consisted of two types: cortisone free alcohol, and 15 mg. pellets of cortisone acetate. The free alcohol form and the compound F were first dissolved in absolute ethanol and then diluted with 0.9 per cent saline to obtain solutions containing 100 μg. per 0.5 ml. The histamine diphosphate (Pfanstiehl Chemical Co.) and the epinephrine hydrochloride (Parke, Davis & Co.) were diluted with saline to contain 50 μg. and 10 μg. per 0.5 ml. respectively. The horse serum used for the foreign protein injections, was obtained from Cappel Laboratories.

**Procedure and Results**

1. **Diurnal Variation**

The following experiment was set up to determine the number of eosinophils normally found in the blood and peritoneal fluid of undisturbed adult male mice throughout the day and night. Nine groups of 12 mice were maintained in individual cages and eosinophil determinations were made every 3 hours. Only one determination of eosinophils of blood and peritoneal fluid was obtained from each animal. The data are shown in figure 1.

It may be seen that there is a marked diurnal variation in the total number of blood eosinophils. A peak of 700 cells per cu. mm. was obtained in the morning and a low of 155 cells occurred in the evening. This was in close agreement with reports of Halberg et al. The peritoneal eosinophils did vary significantly (at the 1 per cent level). However, they did not follow the same diurnal rhythm, nor did they fluctuate as much as the blood eosinophils.

2. **Response to Stress**

Experiments were set up to determine whether eosinophils in the peritoneal fluid would follow the same pattern of response as those in the blood following a mild stress. Twenty-two mice were divided into two groups. One group was subjected to stress 3 hours prior to
the eosinophil determination. This stress consisted of handling the animals to obtain a blood sample from the tail. The second group of mice were undisturbed prior to the eosinophil determination. The data obtained are shown in Table 1.

It may be seen that the stressed mice had a significantly lowered blood eosinophil count, but the peritoneal level was slightly higher. Thus a drop in the number of peritoneal eosinophils does not occur at the time of a fall in the blood eosinophils following stress.

![Graph showing diurnal variation of eosinophil cells in the blood and peritoneal fluid of BBF, mice.](image)

**Table 1.** The Effect of Stress upon the Blood and Peritoneal Eosinophils of Normal Male BBF, Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Eosinophil counts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>Blood: 445 ± 91, Peritoneal fluid: 130 ± 29</td>
</tr>
<tr>
<td>Stress (handling 3 hours prior to eosinophil determinations)</td>
<td>12</td>
<td>Blood: 190 ± 26, Peritoneal fluid: 160 ± 36</td>
</tr>
</tbody>
</table>

* All counts consist of eosinophils per cu. mm. followed by standard error.

3. **Response to Foreign Protein**

In a number of preliminary experiments it was found that repeated intraperitoneal injections of human plasma or horse serum would produce a great increase in the number of eosinophils in the peritoneal cavity (unpublished data). A procedure for raising the number of peritoneal eosinophils was standardized as follows: male mice were injected intraperitoneally a total of four times at intervals of three days with 0.1 ml. of sterile normal horse serum. Four days after the last injection the animals were isolated in separate cages.
and kept in special cabinets with the temperature maintained at 27 C. Three days later the mice were used in various experiments.

A total of 39 mice were pretreated with horse serum and used as controls for subsequent experimental procedures. It was found that the average eosinophil count in the blood was 705 ± 50 and in the peritoneal fluid was 5950 ± 687. Thus the number of blood eosinophils was not significantly higher than the morning level of normal un.injected mice (fig. 1). However, the number of peritoneal eosinophils showed a great increase averaging over twenty-five times higher than the uninjected mice.

4. Response to Adrenal Hormones and Histamine

The presence of large numbers of eosinophils in the peritoneal cavity of protein injected mice, suggested that they might provide an excellent opportunity for the study of the direct action of various materials on these cells. In the first set of experiments, animals which had been previously injected with horse serum, were injected intraperitoneally with saline or 100 μg. cortisol, 100 μg. compound F, 10 μg. epinephrine or 50 μg. histamine di-phosphate dissolved in saline. Three hours later eosinophil determinations were made; the

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Time after treatment in hours</th>
<th>Animals</th>
<th>Eosinophil Count*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 ml. normal horse serum</td>
<td>None</td>
<td>0</td>
<td>9</td>
<td>635 ± 65</td>
</tr>
<tr>
<td>0.5 ml. saline I.P.</td>
<td>0.5 ml. saline I.P.</td>
<td>3</td>
<td>14</td>
<td>140 ± 22</td>
</tr>
<tr>
<td>100 μg. cortisol</td>
<td>100 μg. cortisol I.P.</td>
<td>3</td>
<td>15</td>
<td>110 ± 30</td>
</tr>
<tr>
<td>100 μg. compound F</td>
<td>100 μg. compound F, 0.5 ml. saline I.P.</td>
<td>3</td>
<td>6</td>
<td>70 ± 15</td>
</tr>
<tr>
<td>10 μg. epinephrine</td>
<td>10 μg. epinephrine, 0.5 ml. saline I.P.</td>
<td>3</td>
<td>9</td>
<td>60 ± 13</td>
</tr>
<tr>
<td>50 μg. histamine</td>
<td>50 μg. histamine, 0.5 ml. saline I.P.</td>
<td>3</td>
<td>15</td>
<td>80 ± 15</td>
</tr>
</tbody>
</table>

* All counts consist of cells per cu. mm. followed by standard error.

data are tabulated in Table 2. It may be seen that the blood eosinophils of these animals were markedly lower than in the undisturbed animals, but the eosinophils of the peritoneal fluid were not significantly different. It should be pointed out that these animals were subjected to a double dose of adrenal hormones, those injected and those released by the animals' own glands due to stress. Thus by 3 hours the injections of these materials greatly reduced the number of blood eosinophils, but had little or no effect upon the peritoneal eosinophils.

The next experiment was designed to determine if there was a difference in the eosinophil response within the first 3 hours following a subcutaneous or intraperitoneal injection of 100 μg. of cortisol. A third group of animals received 50 μg. of histamine intraperitoneally. Blood and peritoneal fluid counts were taken at 15 minutes, 1, 2 and 3 hours following the injection. The data are summarized in Table 3.

In all the animals the blood showed an eosinopenia beginning in the first hour and increasing by the second and third hours. The eosinophils of the peritoneal fluid did not show a significant decrease except for an initial drop at 15 to 30 minutes after the injection. This drop may be due to the diluting action of the 0.5 ml. of material injected; however, it does coincide with a simultaneous blood eosinophilia. Recovery occurred by 3 hours. Thus the number of peritoneal eosinophils did not change during a 3 hour period either
 Table 3.—The Effect of Injections of Various Materials on the Eosinophils of Blood and Peritoneal Fluid of Protein Treated Mice

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Time After Treatment</th>
<th>No. of Animals</th>
<th>Eosinophil Count*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 cc. sterile</td>
<td>100 µg. cortisone</td>
<td>0 hr.</td>
<td>39</td>
<td>705 ± 50</td>
</tr>
<tr>
<td>horse serum</td>
<td>subcutaneously</td>
<td>15 min.</td>
<td>8</td>
<td>845 ± 97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min.</td>
<td>8</td>
<td>745 ± 251</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hr.</td>
<td>7</td>
<td>705 ± 96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hr.</td>
<td>9</td>
<td>125 ± 33</td>
</tr>
<tr>
<td>0.4 cc. sterile</td>
<td>100 µg. cortisone</td>
<td>0 hr.</td>
<td>39</td>
<td>705 ± 50</td>
</tr>
<tr>
<td>horse serum</td>
<td>intra-peritoneally</td>
<td>15 min.</td>
<td>14</td>
<td>1040 ± 125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min.</td>
<td>13</td>
<td>820 ± 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hr.</td>
<td>12</td>
<td>500 ± 60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 hr.</td>
<td>13</td>
<td>185 ± 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hr.</td>
<td>13</td>
<td>110 ± 30</td>
</tr>
<tr>
<td>0.4 cc. sterile</td>
<td>50 µg. histamine</td>
<td>0 hr.</td>
<td>39</td>
<td>705 ± 50</td>
</tr>
<tr>
<td>horse serum</td>
<td>intra-peritoneally</td>
<td>15 min.</td>
<td>5</td>
<td>685 ± 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min.</td>
<td>5</td>
<td>755 ± 200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hr.</td>
<td>5</td>
<td>510 ± 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 hr.</td>
<td>6</td>
<td>175 ± 55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hr.</td>
<td>6</td>
<td>90 ± 15</td>
</tr>
</tbody>
</table>

* All counts consist of cells per cu. mm. followed by the standard error.

Fig. 2.—Eosinopenic response in blood and peritoneal fluid following the implantation of a 15 mg. pellet of cortisone acetate.
when the cortisone was injected subcutaneously or when it was injected directly into the peritoneal cavity.

In order to avoid the diluting action of the cortisone solutions, cortisone acetate was implanted into the peritoneal cavity in the form of 15 mg. pellets. Blood and peritoneal eosinophil counts were taken at various intervals. The results are shown in figure 2. Blood eosinophils began to drop by 1 hour, and almost completely disappeared by 3 hours. On the other hand, the peritoneal eosinophils did not show a significant drop until 6 hours after the implantation. The eosinophils did not entirely disappear until 48 hours later.

**Discussion**

This paper describes a technic for obtaining peritoneal fluid samples which can be performed very rapidly and simply without sacrificing the animals. Using this technic, attempts were made to correlate changes in the number of eosinophils found in the peritoneal fluid with those found in the blood following various experimental procedures.

The results obtained from a study of the diurnal fluctuation in the number of eosinophils indicated that there was a great rhythmic variation in the blood eosinophils. These data emphasize the importance of knowing the diurnal levels and of the need for taking blood determinations during the same time of day for the experimental and control groups. With this in mind, our eosinophil determinations were performed, whenever possible, in the morning.

The eosinophils of the peritoneal fluid did not fluctuate as much as the blood eosinophils during the day. Following stress, the number of eosinophils did not decrease in the peritoneal fluid at a time when they were rapidly disappearing from the blood stream. This would tend to suggest that there was not a general lysis of eosinophil cells all over the body.

The results presented in this paper agree with previous reports that repeated injections of foreign proteins produce a local concentration of eosinophils. Very large numbers of eosinophils were found in the peritoneal fluid following four injections of 0.1 ml. of sterile horse serum. The eosinophil counts of the peritoneal fluid were approximately twenty-five times higher following the injection of horse serum. The blood eosinophils did not show a significant increase in numbers following the horse serum. It is possible that if we had carried out the experiments over a longer period of time, an increase in circulating eosinophils would have been noted.

A blood eosinopenia occurred within 3 hours following injections of cortisone, compound F, epinephrine or histamine in animals which had been pretreated with horse serum to raise the number of peritoneal eosinophils. The eosinopenia begins approximately 1 hour after the injection and by 3 or 6 hours very few eosinophils are found in the blood. Thereafter (up to 48 hours or more) very few cells are found as long as the adrenal cortical hormones remain at a high level, e.g., following implantation of a pellet.

The eosinophils in the peritoneal fluid on the other hand, did not show a significant drop 3 hours following the injection of the various hormones. There was an eosinopenia 15 to 30 minutes after the intraperitoneal injections which was presumably due to the diluting action of the material injected. This did not occur when cortisone was injected subcutaneously. A significant drop in the
peritoneal eosinophils did occur 6 to 10 hours following the implantation of a pellet of cortisone acetate. An absolute blood and peritoneal eosinopenia was obtained 24 to 48 hours later. The cortisone seemed to produce essentially the same response whether it was injected intraperitoneally or subcutaneously. The reduction in eosinophils was first seen in the blood and several hours later it occurred in the peritoneal fluid.

Many attempts have been made to determine the mechanism of the eosinopenic response to adrenal hormones. Biggart and others have searched in vain to locate a point of concentration where these cells may be deposited. Although the lungs, spleen and other organs could sequestrate the eosinophils from the blood, there has been no experimental proof that this sequestration is responsible for the observed blood eosinopenia. For example, Bierman and co-workers have shown that the lungs are capable of both removing and adding leukocytes to the circulating blood. Furthermore, eosinophils have been shown to infiltrate into the lungs during an eosinophilia produced by parenteral oil injections. Thus the lungs are capable of removing eosinophils under certain conditions, but it has not been demonstrated that this occurs following adrenal cortical hormone stimulation. The spleen has also been suggested as a site for eosinophil removal, but this has not been confirmed and eosinopenia can occur in splenectomized animals.

Recent experiments in the rat have shown that following cortisone the eosinophils in the peritoneal cavity undergo morphologic changes and give off cytoplasmic fragments which are engulfed by free phagocytes. Thus degenerating eosinophilic forms were found in the peritoneal and pleural fluids and suggest that a destruction of the eosinophils is responsible for the observed eosinopenia. In order to integrate these observations into an over-all theory, the possibility of a direct action of adrenal hormones upon eosinophils must be considered. At the present time the in vitro action of cortisone appears to be controversial. If a direct action does occur in the mouse, one would expect that the destruction of the eosinophils would proceed fastest at the point of highest concentration of the adrenal hormones. In other words, following the implantation of a hard pellet of cortisone into the abdominal cavity, one would expect a lysis first in the eosinophils of the peritoneal fluid, and later a decrease in the eosinophils of the blood. This is certainly not the case in the experiments reported in this paper. The cells disappeared first in the blood, and later in the peritoneal fluid. The eventual disappearance of the cells from the peritoneal fluid could be explained by a migration into the blood stream as well as a local phagocytosis as demonstrated by Padawer and Gordon. Therefore, these data do not support the hypothesis of direct destruction of eosinophils, but indicate that the adrenal hormones must act through some other mechanism. Recent data published by Essellier and Wagner indicate that the eosinopenic response is dependent upon the reticulo-endothelial system. When this system is blocked by trypan blue, the blood eosinopenia did not occur following ACTH injections in the guinea pig. Thus the action of cortisone appears to be upon some other tissue such as the reticulo-endothelial system, which in turn is responsible for the decrease in blood eosinophils. At the present time additional experiments are under way in this laboratory to determine the nature of this eosinopenic response.
SUMMARY

A technic of performing reliable eosinophil determinations on the peritoneal fluid of mice is described. This procedure was found to be rapid and did not necessitate killing the animals.

Simultaneous determinations of the blood and peritoneal eosinophils were made at 3 hour intervals throughout a 24 hour period. A marked diurnal rhythm was found in the blood eosinophils, but little change occurred in the number of eosinophils found in the peritoneal cavity.

During a period of 3 hours following stress, there was a marked blood eosinopenia, but little or no change in the peritoneal eosinophils. Repeated intraperitoneal injections of 0.1 ml. normal horse serum resulted in a great increase in the number of peritoneal eosinophils. These cells appeared to be morphologically identical with the blood eosinophils. In mice which were pretreated with horse serum, relatively large injections of cortisone, compound F, epinephrine and histamine produced a marked blood eosinopenia, but failed to reduce the number of peritoneal eosinophils within 3 hours. The response of eosinophils in blood and peritoneal fluid to cortisone was essentially the same either when it was injected subcutaneously or intraperitoneally.

Implantations of pellets of cortisone acetate in the peritoneal cavity produced a blood eosinopenia beginning within 1 hour and reaching a maximum at approximately 3 hours. The peritoneal eosinophils did not begin to drop until approximately 6 to 10 hours after the implantation.

These data were interpreted as indicating that physiologic doses of cortisone, compound F, epinephrine or histamine do not have a direct lytic action upon the eosinophils of mice.

REFERENCES

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