THE DECREASE of circulating eosinophils under the influence of glucocorticoids is an important phenomenon, not only with regard to its diagnostic use, but also from the standpoint of the underlying basic biologic mechanisms. Most previous experimentation has been concerned merely with one aspect of the problem, that is, with the results of the Thorn test; the reaction of the eosinophils was considered only as an indicator of adrenal function. Despite the fact that a disappearance of circulating eosinophils can be readily provoked, this striking finding that an endogenous substance can cause a transitory aneosinophilia had been only incompletely investigated. Nevertheless, this phenomenon is of great biologic significance, since the complete disappearance of a specific type of blood cell is otherwise not observed, or at least occurs only under most severe pathologic conditions.

Several hypotheses have been brought forth to explain the glucocorticoid eosinopenia: direct destruction of eosinophils in the blood, inhibition of eosinophile myelopoiesis, prevention of release in the circulating blood of eosinophils from extramedullary sources, distributional changes within the blood depots, immigration into the tissues, and fixation of eosinophils at sites of functional demand.

For several years this group has attempted to elucidate this phenomenon by experimental and clinical investigation. As research progressed, single stages of this work have been published. Other more recent findings are reported here for the first time. This paper reviews present knowledge concerning glucocorticoid eosinopenia and advances a new hypothesis to explain this important phenomenon.

Some Special Aspects of Glucocorticoid Eosinopenia

The effect of ACTH measured by the urinary output of 17-ketosteroids increases asymptotically with increasing doses, while the duration of administration also plays an important role. When the adrenal cortex is intact, the eosinopenic effect of ACTH and cortisone is similar. For an accurate analysis of this response of blood eosinophils to glucocorticoids, it is necessary to test cases with high initial eosinophil counts, because in cases with normal counts the eosinophils often disappear completely from the blood during the time of effectiveness of one single dose of glucocorticoid.
In our observations on the effect of glucocorticoids in cases of constant and high grade blood eosinophilia, it was noted that the eosinophils never disappear from the blood during the time of effectiveness of one single application of the hormone\textsuperscript{16}; this observation holds even for very high dosage of ACTH or cortisone.

Under intramuscular administration of several doses of 25 to 50 mg. ACTH given at intervals of six hours, a drop to zero of the eosinophils occurs after approximately twenty-four hours\textsuperscript{16} in cases with a blood eosinophilia of 2000 eosinophils per cu.mm. In cases with an eosinophil count of over 8000 cells cu. mm., aneosinophilia is attained only after two to four days.\textsuperscript{16} In one case with a blood eosinophilia of approximately 2800 cells/cu. mm., an intravenous infusion over a period of twenty hours, with a total of 55 mg. ACTH, induced aneosinophilia only twenty-two hours after the beginning of the infusion.

The behaviour of the blood eosinophils under cortisone is essentially the same as under ACTH, i.e., the time factor plays a more important role than the absolute dosage. The decrease of blood eosinophils in cases with extreme eosinophilia is not significantly greater after a single oral dose of 300 mg., or even 600 mg. cortisone, than after a single dose of 50 mg. Despite such high dosage, complete disappearance of the blood eosinophils does not occur. To obtain an aneosinophilia, it is necessary to repeat the administration of cortisone at intervals of four to six hours, during two to four days, depending on the initial eosinophil count.

During these investigations we were able to ascertain a definite relationship between the level of the eosinophil count and the time interval required for the achievement of complete aneosinophilia.\textsuperscript{15} In addition, we found that specific interrelations exist between the number of blood eosinophils and the percentage of juvenile forms; with increasing eosinophilia, the number of juvenile forms greatly increases (table 1). If, at the beginning of the experiment, one divides the eosinophil population into juvenile and old forms, it can be shown that the time required to induce aneosinophilia is related to the number of juvenile forms present. Since the age of a cell determines its life expectancy, this observation points to the significance of the age distribution of the blood eosinophils in relation to glucocorticoid aneosinophilia. In cases with a normal eosinophil count,

**Table 1.—Relationship Between the Grade of Blood Eosinophilia and the Percentage of Juvenile Forms**

<table>
<thead>
<tr>
<th>Number of eosinophils</th>
<th>Juvenile forms</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute values</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>14,955</td>
<td>5443</td>
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</tr>
<tr>
<td>9978</td>
<td>3604</td>
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<td>19.6</td>
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<tr>
<td>4888</td>
<td>147</td>
<td>3.0</td>
</tr>
<tr>
<td>4644</td>
<td>120</td>
<td>2.6</td>
</tr>
<tr>
<td>1872</td>
<td>155</td>
<td>8.3</td>
</tr>
<tr>
<td>1178</td>
<td>33</td>
<td>2.8</td>
</tr>
<tr>
<td>1011</td>
<td>24</td>
<td>2.4</td>
</tr>
<tr>
<td>511</td>
<td>13</td>
<td>2.6</td>
</tr>
<tr>
<td>511</td>
<td>10</td>
<td>2.0</td>
</tr>
</tbody>
</table>
where the majority of eosinophile granulocytes are older forms, aneosinophilia, can be induced within the time of effectiveness of one single dose of ACTH or cortisone. In cases, however, with a high initial eosinophil count, only the continuous administration of glucocorticoids for two to four days can achieve aneosinophilia.

When analysing the effect of the glucocorticoids on the circulating eosinophils, it should be stressed that their number is only the measurable resultant of numerous influences acting on the eosinophile cell system. The number of circulating eosinophils is determined by the correlations existing between the eosinotactic stimuli (demand at the periphery), the eosinophilopoiesis, the release from the sites of production into the blood, the utilization at the periphery, and the life expectancy of the individual cells of the eosinophil population. All these factors must be taken into consideration when attempting to explain the mechanism of glucocorticoid eosinopenia.

**The Regulating Factors of the Eosinophile Cell System**

Extensive experimental material already exists concerning the significance of the eosinotactic stimuli at the periphery and their action on the eosinophilopoiesis. The time relationship between an experimental eosinotactic stimulus and the reaction of the bone marrow has been accurately defined by Homma’s thorough experimentation. A single application of an eosinotactic substance causes within a few hours a tissue eosinophilia at the site of irritation. Since this local eosinophilia is built up at the expense of the eosinophils in the blood, it induces, at first, a blood eosinopenia. The bone marrow responds to this eosinopenia with an increased release of eosinophils, so that a medullary eosinopenia occurs. This eosinopenia provokes a reactive increase of the eosinophil myelopoiesis, which begins about twelve hours after the stimulus is set. Thus, the blood eosinopenia is compensated and, as soon as the peripheral site of stimulation is saturated with eosinophils, there occurs a blood eosinophilia, which we have named “surplus eosinophilia.” From this moment on, the conditions in blood, tissues, and bone marrow quickly return to their original state (fig. 1).

![Fig. 1.—Schematic representation of eosinophilia in tissues, blood, and bone marrow after a single application of an eosinotactic substance (N = reciprocal number of eosinophils). (From E. Homma.)*](image-url)
Having in mind Homma's work, it is thus possible to understand that tissue eosinophilia may exist either with or without blood eosinophilia. Ringoent\cite{15} points out that local eosinophilia may precede general eosinophilia, the former usually exceeding the latter in degree and frequently disappearing first. Homma\cite{16} has further been able to show that the intensity of these reactions and the timing of their occurrence depend on the intensity of the eosinotactic stimuli.

Little is known about the mechanism of destruction of the eosinophile granulocytes. We have found\cite{17} that a block of the reticuloendothelial system (RES) leads to an increase of the blood eosinophils, which can be explained by an inhibition of the destruction in the RES. One can, therefore, assume that the extent of eosinophil destruction is dependent upon the functional state of the RES.

We were able to confirm Homma's findings by observing the general pattern of reaction of the eosinophile cell system after intramuscular injection of ascaris larvae and plant oils.\cite{18,19} The sequence of adjustments, however, appears clearly only when no other eosinopoietic stimuli interfere, in other words, when the utilization and the normal destruction of the eosinophils remain constant during the whole period of observation. Yet, when acute shifts of equilibrium occur within the eosinophile cell system, other factors must be considered, such as the life span of the eosinophils and the life expectancy of the eosinophil population at the time of the experiment. Although the life expectancy of the individual eosinophil cannot be determined, it is necessarily shorter than its total life span.

Many methods have been devised to attempt a determination of the life span of the eosinophile granulocytes. Most previous investigations are based on methods in vitro and yield widely scattered values. Schürch and co-workers\cite{20} using various methods of blood conservation, found no eosinophils after one day. Conserving blood in Moscow solution and Tyrode solution with heparin, Tzank and Dreyfuss\cite{21} found no eosinophile leukocytes left after two days. Benhamou and Mercier\cite{22} report the disappearance of the eosinophils from conserved blood after three days. Quite different is Osgood's estimate of a life span of eight to twelve days, based on observations in blood cell cultures.\cite{23}

All methods in vitro have the common drawback of producing alterations of the granulocytes, which are highly sensitive to any sort of manipulation. This is well demonstrated by their rapid decay after blood-letting and reinfusion. The most important point, however, is that these methods do not really measure the total life span, i.e. the duration of life of a cell from the moment it is released from the bone marrow until it is disposed of by physiological mechanisms of destruction. They measure at best the life expectancy of the granulocytes, which is very variable and depends upon the average age of the cell population at the time they are withdrawn for observation. As already stressed above, there is a close correlation between the age of the eosinophile cell population and the grade of the blood eosinophilia.

We have devised a method for the determination in vivo of the true life span of the eosinophile cells.\cite{24} This method has been based on the following principle: If an agent can be discovered which provokes within the period of time “a” the disappearance from blood of most of the cells under study, the cellular deficit thus induced is a strong stimulus to hemopoiesis. The increased cellular output elicited by this stimulus compensates the preceding loss within the period of time
"t_1." During this period of regeneration, many more cells are produced than under normal conditions. When their life span comes to an end, during the period of time "t_2," an increased cellular decay must occur, which manifests itself by a diminution in the blood counts. The time interval "b" between the time "t_1," when the influence of the cytopenic agent ceases, and the time "t_2," when the greatest drop in the counts occurs (during the period "t_2" of increased cellular decay), corresponds to the total life span of the cells investigated (fig. 2).

In order for this method to be valid, it must be ascertained that the cytopenic agent does not in any way inhibit hemopoiesis. The initial blood count of the cell studied must be high; then only, the artificially induced cell deficit elicits a hematopoietic stimulus strong enough to induce such a large cellular output within the short period of regeneration "t_1," that it is possible with serial counts, to time accurately their simultaneous decay during the period "t_2." It is also necessary that constant conditions with regard to cellular utilization in the body should exist during the entire period of observation.

This general principle may be applied to the study of the eosinophile granulocytes, by using ACTH which induces aneosinophilia without influencing medullary eosinophilopoiesis. This artificial aneosinophilia is followed during the period "t_1" by a strong "regeneration eosinophilia." At the end of the time interval "b," corresponding to the total life span of the eosinophils, a "compensatory eosinopenia" can be observed during the period "t_2."

In our observations the eosinophils were counted every two hours by Randolph's phloxine method. The amount of ACTH required to bring about complete disappearance of eosinophils from the circulating blood ranged from 175 to 425 mg. It is well known that the effect of ACTH wears off six hours after the last injection. The total life span of the eosinophils corresponds, therefore, to
the time interval between the time "f₁," six hours after the last ACTH injection, and the time "f₂," corresponding to the lowest count during the period of "compensatory eosinopenia" "f₂." Based on our experimental data⁴ we arrive at the estimate that the total life span of the eosinophile granulocytes is six days.

The life expectancy of the eosinophils which have passed into the tissues has been estimated by Essellier and Koszewski⁶ at one to two days. This figure is based on the accurate roentgenologic and hematologic observation of a Loeffler's syndrome, artificially induced by self-infestation with ascaris larvae. In this experiment the eosinophil lung infiltration and the eosinophils in the sputum disappeared within one to two days under the influence of ACTH (fig. 6 and 7).

THE EFFECTS OF THE GLUCOCORTICOIDS ON THE EOSINOPHILE CELL SYSTEM

Effects of Glucocorticoids on the Eosinophile Myelopoiesis

From a technical point of view, the effect of glucocorticoids on the eosinophile myelopoiesis can only be studied in cases with a high grade eosinophilia, since, when the eosinophil count is normal, changes lie within the margin of error of the differential count of the marrow smears. Furthermore, only cases where glucocorticoids display a marked eosinopenic effect are convenient for this investigation. It is also important that the time of observation should be extended over days, not only over hours. Finally, it must be known whether at the onset of an experiment the eosinophil production is constant, increasing, or decreasing.

Only a few observations can be quoted in favour of an inhibition of the medullary eosinophilopoiesis by the glucocorticoids.⁷, ⁸ Most authors noticed no change or even an increase of the eosinophile myelopoiesis⁹—¹⁰ under glucocorticoid influence. Our observations reveal that no evidence exists for a direct effect of ACTH on the eosinophile myelopoiesis.¹¹

In five patients with a constant high grade eosinophilia of approximately 1000 to 15,000 eosinophils per cu.mm., doses of 25 to 50 mg. ACTH were given intramuscularly at six hour intervals, until a total dosage of 200 to 450 mg. was reached.¹² The medullary eosinophilia was not influenced, while a reduction of the blood eosinophil count, down to aneosinophilia, was obtained (fig. 3). It is interesting to note that the blood eosinopenia due to the glucocorticoids, which in these cases lasted for over three days, did not act as an eosinopoietic stimulus, as might be expected according to the general behavior of the eosinophile cell system.

A further proof that ACTH does not influence the eosinophile myelopoiesis is provided by the following observation: if the eosinophile myelopoiesis is already set under intense stimulation before ACTH administration, the increase in number of the medullary eosinophils progresses despite the administration of ACTH. In a self-infestation experiment with ascaris larvae,¹³ for instance, we found that the medullary eosinophilia, which at the onset of eosinophilia, which at the onset of ACTH application was increased to 19 per cent, further increased to 37 per cent despite ACTH, while the eosinophil infiltrations of the lung cleared up completely and the eosinophils disappeared from the blood (fig. 6).

The hypothesis of an inhibition of the eosinophil release from extramedullary sites of eosinophilopoiesis⁵ can be dismissed, as the myeloid origin of the eosino-
FIG. 3.—Action of ACTH on the number of eosinophils in blood and in bone marrow in five cases of constant high-grade eosinophilia (mean values).

phile granulocytes in normal individuals, during the postnatal period, has been adequately demonstrated.41

Direct Effects of Glucocorticoids on the Eosinophils

On the basis of their findings of an eosinolytic effect in vitro of compound E and F, Godlowskii19,20 and Muehrcke31 suggested a direct destruction of the eosinophils in vivo by glucocorticoids. Padawer and Gordon35 arrive at the same conclusions after having observed an increased number of degenerating eosinophilic forms in the peripheral blood, following subcutaneous injection of cortisone. Baldridge1 and Coste6 however, negate any direct eosinolytic effect of these hormones in vitro and Panzenhagen and Speirs85 report no changes in eosinophils in peritoneal fluid of mice after intraperitoneal injection of cortisone. Our investigations also lend no support to the concept that the eosinopenic state may result from actual destruction of eosinophils by adrenocortical hormones.

If, for instance, we accept the idea of a cytolytic effect of the glucocorticoids, it is difficult to understand why the eosinophils in the bone marrow are completely spared (see fig. 3). This could only be explained by the additional assumption of a blood-bone marrow barrier for the glucocorticoids, which is not substantiated by any known fact. Further, the inhibition of the eosinopenic effect of ACTH and cortisone after blockade of the reticuloendothelial system by vital dyes, as described by Essellier and Wagner19 brings additional evidence against direct effects of cortical hormones on the eosinophils.

We have recently been able to show that there is definitely no destruction in vitro of the blood eosinophils by the glucocorticoids.8 Two mg. of various steroids having a known eosinopenic effect in vivo were added to defibrinated or citrated blood samples and incubated for 24 hours. A control with the same blood but without steroid addition was run in parallel with each steroid test. The steroids
### Table 2.—Effect of Compounds E and F on Eosinophils in Vitro

<table>
<thead>
<tr>
<th></th>
<th>Number of experiments</th>
<th>Number of experiments</th>
<th>Eosinophil counts per cu. mm. after:</th>
<th>Eosinophil counts per cu. mm. after:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>0 hours</td>
<td>2 hours</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A. Control experiments without steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defibrinated blood alone</td>
<td>12</td>
<td></td>
<td>1227 (460)</td>
<td>1131 (425)</td>
</tr>
<tr>
<td>Citrated blood alone</td>
<td>12</td>
<td></td>
<td>3233 (1277)</td>
<td>3456 (1481)</td>
</tr>
<tr>
<td>Heparinized blood alone</td>
<td>10</td>
<td></td>
<td>1496 (738)</td>
<td>1455 (731)</td>
</tr>
<tr>
<td>B. Experiments with compounds E and F</td>
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<tr>
<td>Defibrinated blood + Compound E</td>
<td>9</td>
<td></td>
<td>1992 (1138)</td>
<td>1884 (1100)</td>
</tr>
<tr>
<td>Citrated blood + Compound E</td>
<td>9</td>
<td></td>
<td>1677 (145)</td>
<td>1781 (1403)</td>
</tr>
<tr>
<td>Defibrinated blood + Compound F</td>
<td>9</td>
<td></td>
<td>832 (203)</td>
<td>675 (231)</td>
</tr>
<tr>
<td>Citrated blood + Compound F</td>
<td>9</td>
<td></td>
<td>1317 (377)</td>
<td>1241 (304)</td>
</tr>
</tbody>
</table>

* The statistical value, in brackets, is the standard error of the mean, S.E. = \( \sqrt{\frac{S^2}{N(N - 1)}} \). None of the variations in the mean eosinophil counts after 0, 2, 4, 6, and 24 hours, with or without steroid addition, are significant according to the calculation, \( M - M' \) significant when greater than \( 3 \times \sqrt{\frac{S.E.M^2 + (S.E.)^2}{3}} \). This table is based on three hundred and fifty duplicate eosinophil counts.

used were Cortone® and Hydrocortone® (Merck), Cortisone® and compound F (Ciba), and the more soluble preparations, cortisone aldehyde (53R693 Merck) and dehydrocortisone acetate (32R0000 Merck). Blood samples from thirty-six subjects with eosinophil counts ranging from 168 to 17,995 cells per cu.mm. of blood were used for pilot, control, and steroid experiments. A total of seven hundred duplicate counts (three hundred and fifty leukocyte and three hundred and fifty eosinophil counts) was performed with the incubated blood.

It may be seen from the data of experiments without steroids (table 2 A) that the eosinophil counts in defibrinated and citrated blood remained as constant as they did in heparinized blood. Therefore, no heparinized blood was used for the steroid experiments, thus precluding any methodical objections with regard to a possible inhibition by heparin of the glucocorticoid action on the eosinophilic cell suspension.\(^6\)\(^8\) No statistically significant drop of the counts in defibrinated or citrated blood incubated with (table 2 B) or without glucocorticoid has been observed.

This evidence from experiments in vitro speaks against the contention that the eosinopenic effect in vivo of these substances is actually due to a direct eosinolytic mechanism.

**Effects of Glucocorticoids on Distributional Shifts of Eosinophils in So-called Blood Depots and on Eosinophil Migration into Tissues**

Other explanations of the glucocorticoid eosinopenia postulate either distributional shifts of eosinophils in vascular sectors functioning as blood depots or a migration of eosinophils into the tissues. However, the proponents of these hypotheses usually do not state precisely whether the eosinophils are supposed to be stored within the blood vessels or within the tissue spaces of the organs.

Since the lungs can, under certain conditions, function as a blood cell depot,\(^3\)\(^4\) the possibility that the eosinophils could be stored in the lungs was taken into consideration; however, proof of this was not brought forth.\(^7\) Against these hypotheses we may quote our observation that, in a case of experimental Loefllier's
syndrome, the eosinophil infiltration of the lungs completely disappeared under ACTH.\textsuperscript{19}

A storage function of the spleen for the eosinophils was also considered as an explanation for the effect of glucocorticoids.\textsuperscript{27, 32} However, the fact that, under glucocorticoids, the eosinophils do not decrease any more in the splenic vein than they do in the femoral artery\textsuperscript{41} and that, histologically, no enrichment of the spleen with eosinophils has been observed, speaks against this postulate.\textsuperscript{22} The storage of eosinophils in the spleen is rendered quite improbable by observations in splenectomized patients and laboratory animals. Speirs and Meyer\textsuperscript{32} showed that in splenectomized mice, a rapid decrease in the number of circulating eosinophils occurs under glucocorticoid influence, which is similar to the response of intact animals. Essellier, Morandi, and Stein\textsuperscript{13} found that the intramuscular administration of 25 I.U. ACTH to twenty splenectomized patients with normal adrenal function had the same eosinopenic effect as in twenty normal controls (fig. 4).

On the basis of the findings concerning lung parenchyma and spleen, the hypotheses of distributional shifts of eosinophils in vascular sectors functioning as blood depots, or of migration of eosinophils into the tissues, can be discarded as explanations of glucocorticoid eosinopenia.

Effects of Glucocorticoids on Eosinotactic Stimuli

Repeated and painstaking histologic examinations of extensive material are generally required for the exact understanding of the development of tissue eosinophilia as the local expression of an eosinotactic stimulus. However, in

![Graph](Fig. 4.—Behavior of blood eosinophils after intramuscular application of 25 international units of ACTH to twenty splenectomized patients (——) and to twenty normal control persons (---) (mean values).)
cases of the Loeffler type of eosinophil lung infiltration, which consists of conglomerates of eosinophils around ascaris larvae (fig. 5), the time relationships can easily be ascertained by observing roentgenologic changes.

Therefore, an artificial Loeffler's syndrome, provoked by self-infestation with ascaris larvae, provided ideal experimental conditions for investigating the gluco-

Fig. 5.—Ascaris larva of a dimension of 200μ in the lung tissue. The larva is surrounded by a dense eosinophilic infiltration. (The dark cells are eosinophile leukocytes). Romanowski, 460: 1. (From W. Loeffler, A. F. Essellier, and M. E. Macedo.)

<table>
<thead>
<tr>
<th>EXPERIMENTAL DAYS</th>
<th>1</th>
<th>22</th>
<th>5a</th>
<th>25</th>
<th>23</th>
<th>6</th>
<th>12</th>
<th>10</th>
<th>24</th>
<th>6</th>
<th>12</th>
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<td>NOVEMBER</td>
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<td>8</td>
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<td>10</td>
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<tr>
<td>ACTH mg</td>
<td>5a</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td>400 mg in 42 hours</td>
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<td>BLOOD Eosinophils</td>
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<tr>
<td>BONE MARROW EOSINOPHILIA</td>
<td>120</td>
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<td>19.5%</td>
<td></td>
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Fig. 6.—Effect of ACTH on the eosinophile lung infiltrations, the sputum eosinophils, the circulating eosinophils, and the eosinophils in the bone marrow, in an artificial Loeffler's syndrome provoked by self-infestation with ascaris larvae. (From A. F. Essellier and B. J. Koszewski.)
Fig. 7.—Complete regression of the eosinophile lung infiltrations within two days under intramuscular application of 400 mg. ACTH in forty-two hours (a and b). Reappearance of a new infiltration (“Succedan-Infiltrat”) at another place, a few hours after termination of the ACTH effect (c) (compare with fig. 6). (From A. F. Essellier and B. J. Koszewski.)

corticoid effects on tissue eosinophilia. The intramuscular administration of 400 mg. ACTH in nine single doses induced a complete regression of the lung infiltration within two days (figs. 6 and 7 a, b). After discontinuing the ACTH, these infiltrations did not recur. However, a few hours after the effect of ACTH had worn off, a new infiltration appeared in another location (fig. 7 c). This new infiltration, called by Loeffler “succeedan-infiltrat,” is caused by a later invasion of ascaris larvae.

The development of this fresh infiltration points to the fact that immediately after the termination of the ACTH effect the possibility again exists of a transfer
of eosinophils from the blood into the lung parenchyma. The absence of a recurrence of those lung infiltrations which had disappeared under ACTH, can be explained by supposing that ACTH completely abolishes the eosinotactic stimuli.

In view of the fact that glucocorticoids abolish the eosinotactic stimuli, the hypothesis of a fixation of eosinophils at sites of physiologic demand is a very improbable explanation of glucocorticoid eosinopenia, as the physiologic demand is always the expression of an eosinotactic stimulus.

Effects of Glucocorticoids on the Reticuloendothelial System

Numerous authors\(^5\)\(^,\)\(^7\)\(^,\)\(^17\)\(^,\)\(^24\)\(^,\)\(^30\)\(^,\)\(^38\)\(^,\)\(^47\)\(^,\)\(^58\) have shown that the administration of substances stored in the reticuloendothelial system (RES) inhibits this system. Essellier and Wagner\(^13\) observed that the storage of trypan blue is followed by an increase in blood eosinophils, which is explained by a reduced destruction of these cells in the RES. On the other hand, it has been demonstrated that stress\(^50\)\(^,\)\(^55\) or the administration of adrenocortical extracts\(^22\)\(^,\)\(^23\)\(^,\)\(^41\) activate the RES.

Based on these observations, we assumed a relationship between the activation of the RES and glucocorticoid eosinopenia and have been able to confirm this hypothesis in animal experiments.\(^12\)\(^,\)\(^13\) In these experiments, oral administration of 0.625 mg. cortisone per 100 Gm. body weight to ten normal guinea pigs produced an eosinopenic effect lasting twenty-four hours. The average

![Graph showing inhibition of glucocorticoid eosinopenia by blocking the reticuloendothelial system with a vital dye: behavior of blood eosinophils after administration of 0.625 mg. cortisone/100 Gm. body weight, given orally to ten normal guinea pigs (---) and to twenty-four guinea pigs pretreated with trypan blue (-----) (mean values with standard error of the mean).](image)

Fig. 8.—Inhibition of the glucocorticoid eosinopenia by blocking the reticuloendothelial system with a vital dye: behavior of blood eosinophils after administration of 0.625 mg. cortisone/100 Gm. body weight, given orally to ten normal guinea pigs (---) and to twenty-four guinea pigs pretreated with trypan blue (-----) (mean values with standard error of the mean).
maximum drop was minus 59 per cent (±6.4 per cent) (fig. 8). The administration of the same dose of cortisone to twenty-four guinea pigs in which the RES had been previously blocked with trypan blue produced no significant eosinopenic effect (fig. 8). These results show clearly that the eosinopenic effect of 0.625 mg. cortisone per 100 Gm. body weight is markedly reduced by the storage of trypan blue in the RES.

The antieosinopenic effect of the storage of trypan blue can be broken through by administration of massive doses of cortisone. It was found that, after oral administration of 2.5 mg. cortisone per 100 Gm. body weight to fourteen guinea pigs pretreated with trypan blue (a dose which corresponds to about 1700 mg. in man), a significant drop of the eosinophil count occurs, despite the blockade of the RES (fig. 9).12

This observation upholds the opinion, first stated by Selye,50 that the RES is activated by the glucocorticoids, and corroborates the assumption of an increased destruction of eosinophils in the RES under glucocorticoids.

Based upon above experimental facts, i.e. (1) blocking the RES with trypan blue induces eosinophilia, (2) the RES is known to be stimulated by the glucocorticoids, (3) the glucocorticoids induce eosinopenia, (4) the glucocorticoid eosinopenia is inhibited by blocking the RES with trypan blue, and (5) this blockade can be broken through by an overdosage of hormone, it may be concluded that the rate of removal of eosinophils under physiologic conditions, as well as under the influence of exogeneous adrenocortical hormones, is a resultant of the state of the RES and the sum of the eosinopenic stimuli.
A NEW HYPOTHESIS FOR THE GLUCOCORTICOID EOSINOPENIA

These different hypotheses, all of which take into account only partial aspects of the whole problem, give no satisfactory over-all answer for the mechanism of the glucocorticoid eosinopenia. The facts presented by the proponents of each of these hypotheses could not be verified by other authors. In addition, as none of the above mentioned theories take into account all the regulatory factors of the eosinophile cell system, none provides an explanation to all known experimental facts.

Our observations lead us to adopt the viewpoint that the eosinopenic reaction to glucocorticoids is caused both by an inhibition of the eosinophil release from the bone marrow and by an increased destruction of eosinophils in the reticuloendothelial system (RES).

The reaction of the eosinophile cell system to glucocorticoids is characterized by a transitory blood eosinopenia or by an aneosinophilia, whereas the eosinophil myelopoiesis remains morphologically unchanged. Since direct eosinophil destruction has been excluded, the complete disappearance of eosinophils from the blood can only be explained by the assumption of a bone marrow-blood barrier. Without such a barrier between site of formation and blood, a small number of eosinophils should always be present in the blood, even in the event of a maximum increase of the demand at the periphery and of a maximum eosinophil utilization. Theoretically, this bone marrow barrier could well be an inhibition of release, due either to a disturbance of the maturation of the eosinophile myelocytes or to an alteration of the mature eosinophils. Since we have found neither quantitative nor qualitative changes of the eosinophil myelopoiesis under glucocorticoids, a functional inhibition of the release of the mature marrow eosinophils must be postulated.

However, this release inhibition from the bone marrow does not by itself explain the glucocorticoid eosinopenia. Obviously, not only must the eosinophils be prevented from penetrating into the circulation, but also those already present in the blood must disappear. Since they are not destroyed by the glucocorticoids and do not wander into the tissues, nor do they leave the peripheral blood as a result of distributional shifts within the vascular system, it must be concluded that the eosinophils disappear from the blood as a result of their utilization and as a result of their limited life span. The utilization of the eosinophils certainly does not play a great role, since the eosinotactic stimuli are completely eliminated by the glucocorticoids. On the other hand, however, the extent of physiologic destruction of the eosinophils is an important factor. We have shown that the amount of destruction of the eosinophils in the RES, which depends upon the previous functional condition of the RES and upon the life span of the circulating eosinophils, is increased by the glucocorticoids.

The glucocorticoids are involved at two separate levels in the regulatory mechanisms of the eosinophile cell system: at the bone marrow through an inhibition of the release and at the RES through an increase in the extent of destruction of the eosinophils (fig. 10).

Whether a blood eosinopenia or a blood aneosinophilia occurs as a consequence of a single outpouring of hormone from the adrenal cortex, or as the result of a single dose of glucocorticoid, depends upon the age, i.e., upon the life expectancy.
Fig. 10.—Schematic representation of the mode of action of the glucocorticosteroids on the eosinophile cell system.

of the individual elements of the eosinophil population. Since the percentage of young cells with a longer life expectancy is directly correlated with the number of eosinophils present in the blood, so, in final analysis, the original eosinophil count is the deciding factor in the development or nondevelopment of aneosinophilia after a single dose of glucocorticoid. When ACTH or cortisone is administered continuously, the level of the original eosinophilia determines the time lapse before blood aneosinophilia is attained.

**Summary and Conclusions**

This paper deals with basic problems of glucocorticoid eosinopenia and of regulations within the eosinophile cell system.

The effects of glucocorticoids on single regulatory factors of this system, such as eosinotactic stimuli, eosinopoiesis, life span and rate of removal of eosinophils, are discussed in detail. Eosinophiliopoiesis is not influenced by glucocorticoids. This finding, added to the observation that blocking the reticuloendothelial system suppresses the glucocorticoid eosinopenia and that these compounds have no eosinolytic properties in vitro, speaks against the contention that the eosinopenic effect of the glucocorticoids is actually due to a direct lytic effect on the eosinophils. A distributional shift of eosinophils in vascular sectors functioning as blood depots, or a migration into tissues can be abandoned as explanations of glucocorticoid eosinopenia. The hypothesis of a fixation at sites of physiologic demand is certainly not a valid explanation of glucocorticoid eosinopenia, since glucocorticoids suppress the eosinotactic stimuli. Blocking
experiments of the RES show that the rate of disappearance of the eosinophils is a resultant of the functional condition of the RES and of the sum of the eosinopenic stimuli.

On the basis of our investigations we arrive at the conclusion that glucocorticoid eosinopenia depends upon an inhibition of the release of mature eosinophils from the bone marrow and upon an increased destruction of eosinophils in the reticuloendothelial system. The level of the original eosinophilia, that is to say, the percentage of young forms with a long life expectancy, determines the degree of eosinopenia attained by a single application of glucocorticoid and the time lapse before aneosinophilia develops under continuous glucocorticoid administration.

**Summario in Interlingua**

Iste articulo tracta del problemas basic de eosinopenia glucocorticoide e del mechanismo regulatori intra le systema del cellulas eosinophile.

Es discutite in detablo le effecto de glycocorticoides super factores regulatori individual intra iste systema. Tal factores es: stimulos eosinotactic, eosinopoiese, expectation de superviventia de eosinophilos, e rata de disparition de eosinophilos. Eosinopoiese non es afficite per glucocorticoides. Iste constatatation—in conjunction con le observationes que le blocada del sistema reticuloendothelial supprime le eosinopenia glucocorticoide e que iste compositos ha nulle proprietates eosinolytic in vitro—es un argumento contra le conception que le effecto eosinopenic del glucocorticoides es realmente le resultato de un directe effecto lytic de lor parte super le eosinophilos. Pote esser abandonate le idea que eosinopenia glucocorticoide es explicable per un alterate distribution del eosinophilos intra le sectores vascular que functiona como depositos de sanguine o per un migration del eosinophilos verso le texitos. Certo le hypothesis de un fixation del eosinophilos al sitos de demanda physiologic non es un valide explication de eosinopenia glucocorticoide, proque glucocorticoides supprime le stimulos eosinotactic. Experimentos in blocar le sistema reticuloendothelial demonstra que le rata de disparition del eosinophilos es un resultante del condition functional del sistema reticuloendothelial e del summma del stimulos eosinopenic.

Nostre investigationes nos conduce al conclusion que eosinopenia glucocorticoide resulta de un inhibition del lancear de eosinophilos matur per le medulla ossee e de un accelerate destruction de eosinophilos in le systema reticuloendothelial. Le nivello del eosinophilia original, i.e., le procentage de formas juveme con un longe expectation de superviventia, determina le grado de eosinopenia resultante de un singule application de glucocorticoide e le tempore requirite pro le disvelopppamento de aneosinophilia sub le continue administration de glucocorticoides.

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