THE RESPONSE OF EOSINOPHILS IN THE GUINEA PIG TO SENSITIZATION, ANAPHYLAXIS AND VARIOUS DRUGS

By Max Samter, M.D.

I. Review of Early Concepts and Experimental Studies. Scope

THE HISTORY of the eosinophilic polymorphonuclear leukocytes (referred to as 'eosinophils' throughout this study) has few landmarks. In 1846, Wharton Jones1 gave the first reliable description of 'coarse granules' in colorless blood cells. Their morphologic characteristics are so striking that early (Max Schultze2) and recent (Cunningham and Tompkins3) observations differ only in insignificant detail. Paul Ehrlich4 established in 1879—in his 'farbenanalytischen Untersuchungen'—the distinctive mark of the fixed cell; namely, the elective staining of its α-granules with acid dyes.

Since then, innumerable data on the occurrence of eosinophils have been collected. A comprehensive survey prepared by Emil Schwarz5 in 1914 listed in its 653 pages a bibliography of 2738 publications. Yet while Schwarz and others defined the occurrence of eosinophils under various clinical and experimental conditions, they added little to our knowledge of their function. "The association of eosinophilia with a variety of unrelated disorders is still not well understood." (Bethell, Sturgis, Rundles and Meyers6).

Experimental research has been retarded for two reasons. The first reason is the direct result of the controversy about the site of origin of the eosinophils. Authors who regard the bone marrow as their only source, will interpret circulating eosinophils as the necessary link between their site of formation and the tissues in which they are eventually found. Authors who accept the concept of the development of eosinophils at sites other than the bone marrow, have concluded that the same circulating eosinophils represent an overflow, or have been discarded from the tissues in which they have developed.

Ehrlich7 committed himself to the hypothesis that eosinophils are formed in the bone marrow and are distributed by the blood stream. His concept initiated a lively scientific controversy about the question of the homoplasic or heteroplasic origin of eosinophils which fills the early volumes of 'Folia Hematologica.' (Ascoli, Pappenheim, Weidenreich, Maximow, Downey, and Ringoen).

Biggart8 has summarized some of the controversial questions: whether or not blood and tissue eosinophils are identical; whether there is local multiplication of eosinophils; assuming that there is only one type of eosinophil, what causes their emigration from the blood into the tissues; what finally are the relations between bone marrow and tissue eosinophilia?

Several of these questions have been studied by Opie, Schlecht and Schwenker, Weinberg and Seguin, and Homma. The majority of investigators have recognized the bone marrow as the source of the eosinophils which appear in the peripheral circulation, but a final agreement between the dissenting factions has not been reached. Cooke9 has only recently claimed that the discrepancies between the number of eosinophils in blood and tissue are sufficient reason to assume the existence of two different

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RESPONSE OF EOSINOPHILS IN GUINEA PIG

2.18 RESPONSE OF EOSINOPHILS IN GUINEA PIG

types of eosinophils. And while Homma\(^8\) emphasizes the fact that 'no intermediary stages exist between small lymphocytes, large lymphocytes, and macrophages on one side, eosinophils on the other side,' Ringoen\(^9\) concludes: "Tissue eosinophils are derived from various sources; lymphocytes, large mononuclears, plasma cells and adventitial cells being regarded as parent cells.'

In spite of the existing disagreement as to the site of origin of eosinophils, the intimate relationship of eosinophilia to certain phenomena of hypersensitivity has been established beyond doubt (Hajos,\(^2i\) Hajos and Mazgon,\(^22\) Campbell, Drennan and Rettie,\(^26\) and D. H. Campbell\(^24\)). Its mechanism, however, as well as its function and meaning are still obscure. It is surprising to note that experiments of very similar nature have led investigators to diametrically opposed conclusions. Analysis of the literature reveals what must be regarded as the second reason for the hesitant progress of experimental research on eosinophils: a multitude of experimental procedures in a field where minimal changes in technic are bound to cause major changes in results.

It will become necessary, therefore, to apply the rigid standards which distinguish immunologic research in general to research on eosinophils which are part of the immuno-reactions of the organism. Study of eosinophilic response requires a uniform experimental technic which includes a uniform species, a uniform antigen, and a uniform route of administration. Accordingly, the first section of this investigation discusses criteria for the choice of the experimental animal, the choice of a suitable antigen, its dose, and the route by which it is administered.

II GENERAL CONSIDERATIONS

Choice of the Experimental Animal

Review of the literature shows that guinea pigs have been preferred for study of experimental eosinophilia although other species have been investigated. Data about hematologic changes in rabbits and dogs, however, are scanty and controversial. White rats—used by Homma\(^18\) in an attempt to correlate eosinophils in bone marrow, blood and tissues after injection of parasites and parasitic material—have the disadvantage that they are very unresponsive to anaphylactic sensitization. If one intends to demonstrate the allergic state of the sensitized animal by the symptoms of anaphylaxis, guinea pigs are most suitable and were therefore employed.

The guinea pigs were bought at random and raised until they weighed between 400 and 450 grams at the time of the experiment. It appeared advisable to eliminate larger guinea pigs, since Opie\(^15\) in his early studies had shown that guinea pigs tend to develop a spontaneous eosinophilia which increases with increasing weight. The cause of this eosinophilia has not been established although parasitic infection such as by *Megastoma entericum* in the small intestines and *Infusoria* in the cecum has been suspected. The diet consisted of oats supplemented by lettuce and carrots; no added water was given. The average differential count prior to sensitization of the 482 animals used in our studies was as follows: Lymphocytes 42 per cent, neutrophils 55 per cent, monocytes 3 per cent. Eighty-two per cent of the animals had no eosinophils, 14 per cent (68) had 1 per cent. The remaining guinea pigs which had up to 7 per cent eosinophils were classified as a separate group.
They are included and discussed in section VI. The figures at which we have arrived differ slightly from those quoted by other observers; it is felt that this difference may be due, in part, to differences in strains used by this author. The figures listed in Klieneberger’s manual, however, are based on too few animals to serve as a satisfactory base line.

Each experimental group consisted of either six or eight animals, male or female, and no sex distinction was made in our studies.

Choice of the Antigen

Selection of a suitable antigen became an interesting problem since Campbell correlated the insolubility of the antigen as well as the presence of -SH groups with its ability to elicit eosinophilia. The literature is full of curious contradictions. Homma reported that coagulated egg albumen and fibrin failed to produce a significant tissue eosinophilia in white rats. Schlecht, on the other hand, was able to observe (in two experimental animals) ‘‘extraordinary increase in eosinophils’’ after injection of a 2 per cent fibrin solution; neither author gives sufficient data about the preparation of the antigen to permit evaluation of the discrepancies.

We have conducted a considerable number of preliminary experiments in order to establish variations of the response of eosinophils in guinea pigs to antigens of various solubility and antigenicity as expressed by the severity of anaphylactic reactions following re-injection. The antigens tested included fibrin, several fractions of egg white prepared for us by Dr. A. G. Cole, and hapamine (histamine conjugated with a despeciated horse serum globulin through azo-linkage), a compound reported to be a poor antigen (Cohen and Friedman).

Table 1 summarizes some of the results. The animals had been sensitized twenty-one days before the test by intraperitoneal injection of .7 cc. of a 1 per cent solution of the protein under investigation. They were reinjected intracardially with the homologous antigen. Differential counts were taken of the surviving animals three hours and twenty hours after reinjection. The percentage of eosinophils found after twenty hours (when the maximal eosinophilia had been reached) is

<table>
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<th>Table 1.—Choice of Antigen</th>
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<tr>
<td>8 guinea pigs sensitized and reinjected with Ovalbumin. % of eosinophils 20 hours after reinjection</td>
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Percentage of eosinophils (twenty hours after reinjection) in a consecutive series of guinea pigs sensitized and reinjected intracardially with proteins of strong, moderate, and low antigenicity.
listed in table 1. Guinea pigs sensitized and reinjected with ovalbumin show the lowest percentage of eosinophils, but the highest incidence of fatal reactions, while guinea pigs sensitized and reinjected with hapamine develop a considerable eosinophilia in spite of the absence of fatal shock. The anaphylactic reaction as well as the eosinophilic response are phenomena of sensitization, since neither can be elicited by the introduction of a nonspecific protein; it is evident from our experiments, however, that the effectiveness of a given protein in achieving anaphylactic sensitization does not parallel its ability to produce eosinophilia.

The antigen which was finally selected for this study, horse serum, combined reliable anaphylactic antigenicity with satisfactory ability to produce eosinophilia. With few exceptions, therefore, which are labeled as such, horse serum was used as sensitizing and shocking antigen throughout the experiments. One group of six guinea pigs was sensitized and shocked concurrently with each series of experiments. No conclusions were drawn unless fifty per cent of the controls succumbed to fatal anaphylactic reactions.

The animals which were sensitized by intraperitoneal injection of horse serum with few exceptions did not develop eosinophilia prior to reinjection of the specific antigen. Animals which had more than 1 per cent eosinophils prior to reinjection were excluded from the experiments.

Von Pirquet and Schick had suspected that if a large amount of antigen is injected, a portion of it might persist, unaltered, throughout the period of sensitization, combine with the antibodies which have formed during this period and account thus for the symptoms of serum sickness. Since eosinophils appear in the peripheral circulation subsequent to antigen antibody reactions, it seemed conceivable that the injection of a sizeable dose of horse serum might, similarly, cause eosinophilia. However, in a group of six guinea pigs which were given a sensitizing dose of 2.0 cc. of horse serum, none developed eosinophilia prior to reinjection of the specific antigen.

Route of administration

A survey of experimental research on eosinophils indicates that little attention has been paid to the route by which the antigen was administered. It is a common occurrence to find intradermal, intramuscular, intraperitoneal, intravenous and intracardial administration used indiscriminately within the same group of experiments as if the route of administration were of no consequence. Even if one disregards fundamental objections (Heidelberger, Treffers and Freund,) it is quite obvious that reinjection of antigen into a shock tissue, e.g., the skin, with a resulting local antigen-antibody reaction, creates experimental conditions which cannot be compared with, for example, vascular reinjection. Applied to the study of eosinophils, this variety of routes used for the administration of the antigen makes it understandable why the question has not been adequately answered whether the eosinophilic response to reinjection of antigen is due to the antigen per se, the antigen-antibody reaction, the shock syndrome, or the liberation of substances during the immunologic processes. The experiments of Weinberg and Seguin are a case in point. The results of their observations are significant for
certain phases of the mechanism of the eosinophilic response which will be dis-
cussed later; but they also demonstrate clearly that results obtained after sub-
cutaneous, intraperitoneal, and intravenous reinjection are not comparable.

Although it is possible to sensitize guinea pigs by any parenteral route, the
intraperitoneal injection is not only most convenient but known to give, for this
particular antigen, consistent anaphylactic sensitization. The reason for this is
not fully understood although it has been suggested that the copious lymphatic
drainage of the peritoneum, and a resulting efficient elaboration of antibodies,
might be responsible. The sensitizing dose of the antigen, therefore, has been given
intraperitoneally.

The route of readministration on the other hand can influence the outcome of
the experiments in several ways. The speed of absorption of reinjected protein
varies with the site of administration. The site of the injection determines, there-
fore, the time interval between the introduction of the antigen and the changes
which it causes. If, furthermore, reinjection is made into sensitized shock tissue,
e.g., the skin, it becomes questionable how much non-neutralized antigen reaches
the rest of the animal. Accordingly, Weinberg and Seguin, who reinjected the
majority of animals intravenously and intraperitoneally, came to conclusions
almost irreconcilable with those of Hajos who administered the antigen in-
tramuscularly or by inhalation. The intravascular reinjection affords an immediate
distribution of the antigen throughout the animal and prevents its retention at the
site of administration. Intracardial rather than intravenous administration was
used by us because of its technical advantages; it requires, however, that a post-
mortem examination be done on each animal which dies during or shortly after the
injection, since the occasional perforation of the heart muscle with massive
hemorrhage might simulate the asphyctic death of acute anaphylaxis.

Technic of the Eosinophil Count

The technic of preference for the study of eosinophils is the absolute eosinophil
count (Zappert, Discombe, and Randolph)

Unfortunately, the amount of blood which can be obtained for repeated studies
from the ear veins of the guinea pig is not sufficient to make absolute blood counts
a routine procedure. This fact has disturbed various investigators (Opie, but
it has been shown that the changes in total white count during this particular type
of experiment are not of sufficient magnitude to discredit the significance of
findings based on changes in differential count alone. Reliability of differential
counts, however, depends on uniform technical handling. The blood has to be
transferred from the ear without pressure and is spread evenly into a thin film be-
tween two coverslips. Even under the most favorable circumstances, this is not
always successfully accomplished. In our experiments a considerable number of
preparations had to be discarded because distribution or preservation of leukocytes
proved unsatisfactory. The coverslips were stained with Wright’s stain and, after
drying, attached to a labeled slide with Canada Balsam. Blood counts taken before
reinjection of antigen and at fifteen, thirty, forty-five, and sixty minute and then
at hourly intervals for twenty-four hours after reinjection of antigen, demon-
strated that there is a time lag between administration of antigen and cellular response. The maximal eosinophilia was not reached before a lapse of twelve to twenty-four hours after reinjection. This observation, found in early work and re-emphasized by Hajos, is of great theoretical interest and has not received the recognition which it deserves.

III. RESPONSE OF SENSITIZED GUINEA PIG TO REINJECTION OF SPECIFIC ANTIGEN

Experimental problem. It is difficult, if not impossible, to produce uniform sensitization in any given group of experimental animals. This difficulty is well known and represents one of the most serious obstacles to quantitative evaluation of results. Rich, for instance, in his studies on the pathogenesis of rheumatic fever and periarteritis nodosa states: 'This native, individual difference in reactivity, which not only determines whether a given sensitized individual will, on contact with the antigen, develop a hypersensitive reaction but also determines in what tissue the hypersensitive reaction will occur, has, in all probability, an hereditary, constitutional element.' The variation in the eosinophilic response is even greater than the differences in anaphylactic reactivity; we have no indication that the mechanisms of either are related. Our experimental procedure ascertains within reasonable limits the anaphylactic antigenicity of the antigen used in each particular series of experiments. The ability of the same antigen to elicit an eosinophilic response in sensitized guinea pigs after intracardial reinjection remains to be established before an analysis of factors which influence such response can be attempted.

Experimental procedure. Thirty-six guinea pigs were sensitized by intraperitoneal injection of 1 cc. of horse serum without preservative. Twenty-one days later, 28 guinea pigs were reinjected with 0.75 cc. of horse serum intracardially. The 8 remaining guinea pigs were injected with 0.75 of a 1 per cent solution of crystallized ovalbumin. Differential counts were obtained of all animals prior to reinjection, of the surviving animals three hours and twenty hours after reinjection.

Results. The percentage of eosinophils observed twenty hours after reinjection of horse serum in a series of 12 guinea pigs is listed in column 1, table 2. None of the guinea pigs had eosinophils prior to reinjection. Subsequent to reinjection, the figures range from 0 to 26 per cent. Only one animal failed to show an eosinophilia. Only 2 out of the 8 guinea pigs injected with crystallized ovalbumin developed an eosinophilia of 2 per cent and 3 per cent respectively; differential blood count of the remaining 6 guinea pigs showed the changes in lymphocytes which are known to follow the injection of protein, but no eosinophils. We have omitted the three hour counts from the table, since they do not add any essential information. Their possible significance will be discussed in Section V.

Conclusions. Preliminary experiments summarized in table 1 had shown that the nature of the antigen determines, in part, the response of the eosinophils in the sensitized and reinjected guinea pig. Response is independent of severity of anaphylactic symptoms. Regardless of its extent, however, response depends in all cases on reintroduction of the specific antigen. Injection of a nonspecific protein in sensitized guinea pigs does not result in eosinophilia. The response also varies
TABLE 2.—Percentage of Eosinophils Twenty Hours after Reinjection of Horse Serum in Horse Serum Sensitive Guinea Pigs

None of the animals had eosinophils prior to reinjection. Twelve guinea pigs unprotected, 54 guinea pigs protected by various antihistamine drugs.

Sixty-four horse serum sensitive guinea pigs. Thirty minutes before intracardial readministration of horse serum, injection of

<table>
<thead>
<tr>
<th>No protective drug (12 animals)</th>
<th>Benadryl 5 mg./Kg. (12 animals)</th>
<th>S.Y.14 0.15 mg./Kg. (12 animals)</th>
<th>S.Y.18 1.5 mg./Kg. (12 animals)</th>
<th>S.Y.27 0.15 mg./Kg. (8 animals)</th>
<th>S.Y.28 0.15 mg./Kg. (8 animals)</th>
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Averages and standard errors

| 8.50 ± 1.81 | 6.75 ± 1.50 | 10.6 ± 2.56 | 11.3 ± 1.91 | 8.88 ± 2.51 | 6.88 ± 1.40 |

Table 2a.—Percentage of eosinophils in guinea pigs sensitized to, but not reinjected with, horse serum, twenty hours after intraperitoneal injection of Benadryl and S.Y. 14

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<th>Benadryl</th>
<th>S.Y.14</th>
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IV. Response of the Sensitized Guinea Pig Protected by Antihistamine Drugs to Reinjection of Specific Antigen

Experimental problem. Introduction of antihistamine drugs, the clinical significance of which is still under investigation (Feinberg4), has been instrumental in widening the scope of investigative work in allergy. It permits the study of animals of maximal sensitivity which, thus protected, survive reinjection of the specific...
RESPONSE OF EOSINOPHILS IN GUINEA PIG

antigen. Synthesis of compounds which combine antihistaminic and sympatholytic action has made it possible to re-examine the concepts of workers who, like Hajos, emphasize the prominence of the autonomic nervous system in eosinophilia. We wish to state that this part of our study would have been incomplete, if not impossible, without the advice and the generosity of Dr. E. R. Loew who not only supplied the necessary chemicals and the data on their comparative potency, but also consented to integrate our preliminary and final experiments on the response of the eosinophils into his own which tested the antianaphylactic action of the drugs.

The first series of experiments was carried out on animals protected with β-dimethylaminoethyl benzhydryl ether-HCl: benadryl.

\[
\text{HC} \quad - \quad O-\text{CH}_2\text{CH}_2\text{N} \quad \text{HCl} \\
\text{CH}_3 \quad \text{CH}_3
\]

The potency of this substance is about one-twentieth of a comparable French antihistamine drug, neoantergan (N-p-methoxybenzyl-N-dimethylaminoethyl aminopyridine).

In subsequent experiments, a number of alkyl derivatives of α-naphthyl-methyl-β-chloroethylamine and of α-bi-phenoxylethyl-β-chloroethylamine were used.* Achenbach and Loew have shown that the histamine antagonism produced by these compounds is of an order similar to the one afforded by benadryl and its relatives, but while the latter enhances the pressor response to epinephrine in animals, the former exert epinephrine blocking action. They reverse, for instance, the pressor effect of epinephrine in dogs. The following compounds were used and they are designated by their test numbers:

SY 14: α-naphthylmethyleneethyl-β-chloroethylamine HCl

\[
\text{HC} \quad \text{C-N} \quad \text{CH}_3\text{CH}_2\text{Cl} \cdot \text{HCl} \\
\text{C}_9\text{H}_8 \quad \text{C}_9\text{H}_8
\]

SY 28: α-naphthylmethyleneethyl-β-bromoethylamine HBr

\[
\text{HC} \quad \text{C-N} \quad \text{CH}_3\text{CH}_2\text{Br} \cdot \text{Hbr} \\
\text{C}_9\text{H}_8 \quad \text{C}_9\text{H}_8
\]

*Those compounds were synthesized and made available to us by Drs. G. Rieveschl, Jr., R. Fleming and W. R. Coleman of Parke, Davis and Company, Detroit, Michigan.
SY 27: α-naphthylmethylisopropyl-β-chloroethylamine·HCl

SY 18: β-2-biphenyloxyethyl-β-chloroethylamine·HCl

Antihistamine activity of SY 14, SY 28 and SY 27 equals approximately that of neoantergan, while SY 18, like benadryl shows only one-twentieth of the protective action produced by neoantergan.

Experimental procedure. Eighty guinea pigs were sensitized with horse serum in accordance with the technic described in Section II. Twenty-one days after the sensitizing injection, they were divided into seven groups. The first group (15) was given benadryl, 5 mg./Kg. in aqueous solution, thirty minutes prior to reinjection. The second group (15) was given SY 14, 1.15 mg./Kg. in aqueous solution, thirty minutes prior to reinjection. The third group (15) was given SY 18, 1.5 mg./Kg. in aqueous solution, thirty minutes prior to reinjection. The fourth group (10) was given SY 27, 1.15 mg./Kg. in aqueous solution prior to reinjection. The fifth group (10) was given SY 28, 1.15 mg./Kg. in aqueous solution thirty minutes prior to reinjection. The sixth group (10) was given benadryl, 5 mg./Kg. in aqueous solution, without subsequent reinjection of horse serum; the seventh group (10) was given SY 14, 1.15 mg./Kg. in aqueous solution without subsequent reinjection of the antigen. The antihistamine drugs were given subcutaneously or intraperitoneally. The last two groups were included in order to establish whether antihistamine drugs per se had any influence on the differential count of the guinea pig. Doses used were suggested by Dr. Loew and provided satisfactory protection against fatal anaphylactic reactions. It may be noted that the same dose was used for SY 14, SY 28 and SY 27. Quantitative studies would have to make allowance for the higher molecular weight of the bromide. Blood counts were taken three hours and twenty hours after reinjection of horse serum, or, in the last two groups, after injection of the antihistamine drug.

Results. The percentage of eosinophils observed twenty hours after reinjection of horse serum in sensitized guinea pigs which were protected by five different antihistamine drugs against fatal anaphylactic reactions is listed in columns 2, 3, 4, 5, and 6, Table 2. The corresponding figures for the two groups of sensitized guinea pigs
pigs which were injected with antihistamine drugs only, without subsequent re-
introduction of the antigen, are found in table 2a. The number of animals reported
is somewhat lower than the number of those sensitized. This is due to loss of
animals which for various reasons, died during or after sensitization.

The most outstanding findings in this series of experiments is the fact that the
antihistamine drugs, while they block the anaphylactic reactions in the sensitized
and shocked guinea pig, do not abolish the eosinophilic response. If normal
distribution of the eosinophilic response could be assumed (which in view of our
data is not justified) one could calculate that any difference which exists in eosino-
philic response of animals protected by various antihistamine drugs is obliterated
by the exceedingly high variability of such a response. This is obvious in the
averages and standard errors listed in table 2a. Other types of distribution which
have been tested lead to identical conclusions. Nevertheless, statistical analysis
does not deny the possibility that drugs which combine histamine antagonism
with sympatholytic action, enhance the eosinophilia found in the peripheral
blood twenty hours after reinjection of the antigen in sensitized guinea pigs. The
administration of benadryl alone, without subsequent reinjection of the antigen,
produced only minor changes in three out of six animals; the percentage of eosino-
phils observed after administration of SY 14 without subsequent reinjection of
antigen remained below the average of reinjected guinea pigs, but showed suf-
cient increase to make further discussion necessary.

Conclusions. It has been repeatedly stated that antihistamine drugs represent a tool
to "test, extend, or restrict the histamine theory of allergy" (Mayer37), in which
eosinophils have been included (Code8). Ratner9 maintains that validity of the
histamine concept has yet to be established. However, the fact that eosinophilic
response is not abolished by antihistamine compounds lends itself to several
interpretations.

If eosinophilic response is not due to release of histamine, it could not be ex-
pected to be altered by antihistamine drugs. If, on the other hand, liberation of
histamine or histamine-like substances was the direct cause of appearance of
eosinophils in the circulation, it must be assumed that antihistamine drugs fail to
act on the underlying mechanism. This is conceivable, since various effects of
histamine, e.g., its action on gastric secretion, are not uniformly influenced by
antihistamine drugs. In the latter case, one could expect that eosinophilic response
in protected animals would exceed the response in unprotected animals since more
histamine—blocked from its pharmacologic action on smooth muscles and capil-
laries—might be available. It might be reasoned—paradoxical as it seems—that
antihistamine drugs should exaggerate those effects of histamine against which
they do not protect. As a matter of fact, the administration of antihistamine drugs
alone might cause such effects as soon as the amount of histamine which they
displace and divert exceeds the physiologic threshold; it is this consideration which
led us to study the action of antihistamine drugs per se without subsequent re-
injection of antigen on the eosinophils in the guinea pig.

The mechanism of histamine activity, i.e., its rapid change from an inactive into
an active form, is insufficiently understood. By the same token, the action of anti-
histamine drugs has not yet been explained. We are aware that our findings that antihistamine drugs do not abolish the eosinophilia which follows an antigen antibody reaction in the guinea pig neither confirm nor disprove the possibility that histamine participates in its development.

The action of antihistamine drugs with sympatholytic activity is of interest in view of the known fact that epinephrine decreases or abolishes an existing eosinophilia (Schwenker and Schlecht19), or reduces the eosinophilia which follows reinjection of specific antigen (Campbell22). Campbell has concluded from his experiments that epinephrine inhibits eosinophilia because it eliminates the shock syndrome without interfering with the antigen antibody reaction itself. The same statement, however, applies to antihistamine drugs which have no inhibiting effect on eosinophilic response. We feel that our concept of the importance of shock in the mechanism of the eosinophilic response requires a careful analysis and, probably, revision.

V. ROLE OF SHOCK IN PRODUCTION OF PERIPHERAL EOSINOPHILIA

Experimental problem. The importance of shock in the mechanism of the eosinophil response has been suggested by many authors, notably by Hajos21 who claimed that an agitation of the autonomous nervous system, “vegetative Erschütterung,” is required before eosinophils appear in the peripheral circulation. E. von Neusser11 had described eosinophilia following pilocarpine injection as early as 1892; Bertelli, Falta and Schweiger42 demonstrated the effect of epinephrine (decrease in eosinophils) as well as that of pilocarpine and choline (increase in eosinophils), and attributed the results to action of the drugs on sympathetic and parasympathetic nerves respectively. Camp43 found, however, that both parasympathetic and sympathetic stimulation raised the number of eosinophils in rabbits which makes the existence of a specific neural regulation rather unlikely.

Jajos21 had concluded that the injection of foreign protein stimulates the formation of eosinophils in the bone marrow, but that an “autonomic shock” would force their release into the peripheral circulation. We have always felt that this concept of “shock” was too vague to support his theory convincingly. In order to test its validity we have grouped the experimental animals which survived the reinjection of the homologous antigen according to severity of “shock” symptoms. The groups were labeled as follows:

1. Severe shock followed by convulsions, collapse and coma: ++++
2. Sneezing, marked dyspnea, urination and defecation: ++
3. Chattering teeth, ruffled fur, excitement without respiratory symptoms: +
4. and 5. Very slight, indefinite, or no symptoms: ± or 0

The results are found in table 3. In addition to our own findings, we have re-examined the figures published by Weinberg and Seguin; they list the percentage of eosinophils observed in the blood of guinea pigs sensitized with horse serum, twelve to twenty-four hours after subcutaneous or intraperitoneal reinjection. Table 3a lists those guinea pigs which had no eosinophils prior to reinjection and are, therefore, comparable to our own; the symptoms were “marked,” but no description in detail was given, and we have assumed that they correspond roughly
RESPONSE OF EOSINOPHILS IN GUINEA PIG

to our second, ++, group. Table 3b lists those of Weinberg and Seguin's animals which had eosinophils ranging from 4 to 17.6 per cent in the peripheral blood.

**Table 3.—Severity of Shock Symptoms and Eosinophilia (55 guinea pigs)**

<table>
<thead>
<tr>
<th>Severity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>++++</td>
<td>Severe shock followed by convulsions, collapse, coma.</td>
</tr>
<tr>
<td>++</td>
<td>Sneezing, marked dyspnea, urination, defeation.</td>
</tr>
<tr>
<td>+</td>
<td>Chattering teeth, ruffled fur, excitement without respiratory symptoms.</td>
</tr>
<tr>
<td>± o</td>
<td>Very slight, indefinite or no symptoms.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Route</th>
<th>Before reinjection</th>
<th>After reinjection</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>4 8.3 s.c.</td>
<td>18 s.c.</td>
<td>none</td>
</tr>
<tr>
<td>i.p.</td>
<td>5 3 18 27.6 i.p.</td>
<td>14 i.p.</td>
<td>++</td>
</tr>
</tbody>
</table>

Percentage of eosinophils (twenty hours after reinjection) of guinea pigs sensitized and reinjected with horse serum. None had eosinophils prior to reinjection.

**Table 3a.—(Compiled from Weinberg and Seguin)**

<table>
<thead>
<tr>
<th>Shock symptoms and eosinophils</th>
<th>No symptoms</th>
<th>++</th>
<th>+</th>
<th>±</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6</td>
<td>0</td>
<td>4</td>
<td>18</td>
<td>14</td>
<td>17.6</td>
</tr>
<tr>
<td>4.3</td>
<td>6.6</td>
<td>8</td>
<td>12</td>
<td>6</td>
<td>6.6</td>
</tr>
<tr>
<td>3.3</td>
<td>8.3</td>
<td>4</td>
<td>14</td>
<td>6</td>
<td>6.6</td>
</tr>
<tr>
<td>2.6</td>
<td>2.3</td>
<td>15.6</td>
<td>14.3</td>
<td>14.6</td>
<td>14.6</td>
</tr>
<tr>
<td>17.3</td>
<td>8.3</td>
<td>27.6</td>
<td>21.6</td>
<td>12.6</td>
<td>14.6</td>
</tr>
<tr>
<td>12.3</td>
<td>18</td>
<td>14</td>
<td>12</td>
<td>6</td>
<td>12.6</td>
</tr>
<tr>
<td>2</td>
<td>5 3</td>
<td>14</td>
<td>14</td>
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<td>14.6</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Percentage of eosinophils (twelve to twenty-four hours after subcutaneous or intraperitoneal reinjection) in guinea pigs sensitized and reinjected with horse serum. Percentage prior to reinjection: less than one per cent.

prior to reinjection of horse serum. We have eliminated from our experiments animals which showed an eosinophilia prior to the "shocking" injection because the introduction of another variable factor would further complicate the already
difficult interpretation. In this instance, however, table 3b illustrates well the point in question: of the 12 animals listed, 5 had marked symptoms of shock, 7 had none; the fact that only the intraperitoneal, not the subcutaneous, reinjection produced symptoms is of course a phenomenon with which investigators have long since become familiar.

**Results and conclusions.** The tables are self-explanatory and the conclusions are evident. We have been unable to demonstrate any correlation between intensity of shock symptoms and appearance of eosinophils in the peripheral circulation of guinea pigs sensitized and reinjected with horse serum. Our findings compare well with those established by previous authors in experiments which were conducted for different reasons, but employed a technic comparable to ours. They are also in accord with the findings summarized in table 1, where reinjection of hapamine produced a more pronounced eosinophilia in sensitized guinea pigs than ovalbumin, although the anaphylactic symptoms caused by reintroduction of hapamine were much less severe than those which followed the reinjection of ovalbumin.

VI. **Response of Nonsensitized and Sensitized Guinea Pigs to Injection of Substances Liberated During Antigen-Antibody Reaction: Histamine Phosphate, Heparin, Adenosine**

**Experimental problem.** It had been recognized early that while homologous antigen, protein, is instrumental in the course of events which result in peripheral eosinophilia, it is not its direct cause. Schlecht had examined the ability to produce eosinophilia of a considerable number of substances including leucine, alanine, phenylalanine, glycocoll, asparagine, and also sugar, starch and olive oil: all of these failed to produce an eosinophilic response. In view of the complexity of the literature on the subject, it seems necessary to point out that the chemicals which have been tested fall into two categories: those which act, and those which fail to act on vasomotor regulatory mechanisms. It is important to distinguish clearly between the two groups; a considerable portion of described changes might be attributed to a shift in distribution of the corpuscular elements of the blood. Dobreff, Doitschineff and Marinoff have coined the term "Verteilungsleukocytose" for this phenomenon, and—to name a practical application—we suspect that Vaughan’s so-called leukopenic index might be explained on a similar basis. Drugs which stimulate or inhibit autonomic nerves, belong to the first group, e.g., epinephrine, atropine, physostigmine, acetylcholine, histamine; substances like sugar or cysteine belong to the other.

The distinction which we have just outlined is important for analysis of factors which participate in development of peripheral eosinophilia. It is possible that the eosinophilic response is controlled by vascular mechanisms, e.g., the afferent and efferent circulation of the bone marrow. Part of the experimental evidence points in this direction. Yet, there are other possibilities to be considered. It is conceivable that the eosinophilic response is due to chemical action on a specific enzyme system; vascular factors might, secondarily, control its intensity. The anaphylactic reaction is complex and involves a variety of biologic changes, such as the contraction of smooth muscles in shock tissues, the decreased coagulability of the
blood, the eosinophilic response. Dragstedt has concluded that separate substances are responsible for at least two of these manifestations: "The evidence must be considered conclusive that a tissue liberation of histamine, of heparin, and possibly of choline occurs during the anaphylactic reaction in various animals. In the dog the liberation of heparin can completely account for the incoagulability of the blood and there is no reason to doubt that it may be found in other animals."

Accordingly, the eosinophilic response again might be caused by a different compound which is not yet defined. It is possible that the distinction which we have suggested will facilitate its eventual identification.

The compounds mentioned by Dragstedt appear subsequently to the antigen antibody reaction. Since most of the substances occur under physiologic conditions in the experimental animal, pathologic changes will not result unless the amount injected exceeds the threshold of physiologic balance. We are unable to predict this threshold with regard to the eosinophilic response; accordingly, conclusions must be restricted to the route and the amount used in each experiment.

As far as we have been able to ascertain, no previous studies have been made of the effect on eosinophilia of adenosine and heparin. There is evidence that either might be released during anaphylactic reactions (Rocha e Silver). Adenosine has a rather weak dilating effect on peripheral arterioles; but "administered subcutaneously, [it] causes a migration of leukocytes to the site of injection, an effect which is not produced by histamine" (Best and Taylor).

Experimental studies by Campbell demonstrated that guinea pigs which were given 0.5 mg. of histamine, three times a day for three consecutive days, showed no increase in the percentage of eosinophils during five days following the first injection. He found, however, that guinea pigs sensitized and reinjected with ascaris keratin responded with a more marked increase in eosinophils when the reinjection of the antigen was followed by a series of histamine injections as previously described. The same effect could be obtained, however, if acetylcholine and cysteine, instead of histamine, were used. It is difficult, therefore, to interpret Campbell's findings. He used substances of different biologic activity and we cannot think of any common denominator. The use of antihistamine drugs in our experiments made it possible to inject an amount of histamine which was comparable to the level of histamine or histamine-like substances liberated during anaphylactic reactions, an amount which would be fatal without this protection; Campbell, in his experiments administered histamine intraperitoneally and did not describe any symptoms of shock such as would have to be expected if similar doses were given intracardially.

If nonantigenic compounds fail to call forth an eosinophilic response, the negative results might be due to the ineffectiveness of the given chemical agent, or to the fact that the injected animal was one of those incapable of producing eosinophilia no matter what the stimulus. We will have to rule out the latter possibility by reinjection of the specific antigen subsequent to the injection of the compound under investigation. Exceptions to this rule would be permissible only if it were possible to obtain a strain of guinea pigs which would afford, under standard experimental conditions, a uniform eosinophilic response. We have
discussed the problem with Dr. D. H. Campbell, whose work seemed to indicate that his experimental animals responded more homogeneously than ours to sensitization and reinjection with a variety of antigens. Dr. Campbell was kind enough to supply us, for breeding purposes, with several animals from his strain which has been inbred for more than five years. We intend to study their response as soon as a sufficient number of animals are available.

Experimental procedure. Guinea pigs (32) were sensitized with horse serum in accordance with the technic outlined in Section II. Twenty-one days after the sensitizing injection, they were divided into three groups. The first group (8) was injected with adenosine, 1 mg. in 0.5 cc. of distilled water, intracardially. The injection of adenosine was followed seventy-two hours later by the intracardial reinjection of 0.75 cc. of horse serum. The second group (8) was injected with heparin, 1 mg. in 0.5 cc. of distilled water intracardially; the injection of heparin was followed seventy-two hours later by the intracardial injection of horse serum as described before. The third group (16) was injected with histamine phosphate, 0.5 mg./Kg. in 0.5 cc. of distilled water intracardially; 1 mg. of this solution represents 0.36 mg. histamine base. The injection of histamine phosphate was followed

<table>
<thead>
<tr>
<th>Guinea Pig No.</th>
<th>Intracardial inj. of adenosine, % of eosinophils</th>
<th>Intracardial inj. of heparin, % of eosinophils</th>
<th>Intracardial inj. of histamine, % of eosinophils</th>
<th>Intracardial inj. of horse serum, % of eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bef. inj.</td>
<td>3 hrs. after inj.</td>
<td>20 hrs. after inj.</td>
<td>bef. inj.</td>
</tr>
<tr>
<td>108</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>111</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>363</td>
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<td>385</td>
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</tr>
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<td>7</td>
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<td>4</td>
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<td>362</td>
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<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>417</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>449</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>453</td>
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<td>2</td>
</tr>
<tr>
<td>456</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>
seventy-two hours later by the reinjection of 0.75 cc. of horse serum intracardially as described before. At the same time, a group of nonsensitized guinea pigs (12) were given histamine phosphate, 0.5 mg./Kg. in distilled water, intracardially.

Differential counts were taken before and three hours and twenty hours after each intracardial injection. The animals were given benadryl, 5 mg./Kg. in aqueous solution, intraperitoneally, thirty minutes prior to the injection of histamine phosphate and horse serum; adenosine and heparin were injected without protection. Benadryl was selected as the antihistamine drug for this series of experiments, because we had previously shown that, per se, it failed to increase the percentage of eosinophils in the sensitized guinea pig.

Results. The results of the injections of adenosine, heparin and histamine followed by the reinjection of horse serum are listed in table 4. Adenosine, in the amount injected did not produce an increase in eosinophils; the increase observed after the injection of heparin is not significant enough to warrant conclusions; it might be caused, for instance, by a minute amount of impurities which adhere even to highly purified preparations.

A considerable number of animals injected with histamine died immediately after or during the first twelve hours following the injection. There was, however, no apparent difference in tolerance between the non-sensitized and sensitized group. The non-sensitized animals had a differential count of 0, 0, 1, 2, 3 and 6 per cent eosinophils, respectively, twenty hours after the intracardial injection of histamine phosphate. The percentage of eosinophils in the sensitized group range from 0 to 17 per cent; 6 of the 8 animals, however, which are included in this group, had an eosinophil count of 5 per cent or more.

The reinjection of horse serum makes it evident that animals No. 417 and No. 449 must be eliminated from the series for both failed to respond to the subsequent reinjection of the specific antigen. Animal No. 361 has been included in order to emphasize the fact that the response of the guinea pig to any given substance is obscured if an eosinophilia is present prior to the experiment; this is true even if the response to subsequent reinjection of the specific antigen is satisfactory.

Table 4 also lists the differential eosinophil count obtained three hours after intracardial injection. We are unable as yet to explain the variation in speed with which the maximum percentage of eosinophils is reached. Animals No. 359 and No. 362, for instance, had 3 per cent eosinophils after three hours, but the former showed 17 per cent, the latter only 5 per cent after twenty hours. Similarly, the return to the pre-experimental level varies considerably. The behavior of the eosinophils in animals which showed an eosinophilia prior to the reinjection of the specific antigen, presents another interesting aspect of the same question. We have listed, in table 5, two groups of guinea pigs sensitized and reinjected with horse serum. The first group had not more than 1 per cent, the second group from 2 to 7 per cent eosinophils before the shocking injection of horse serum was given. A comparison of the percentage of eosinophils observed in each group three hours and twenty hours after reinjection, makes us suspect that a balance develops between an initial disappearance and a secondary reappearance of eosinophils in the
Differential counts at short intervals might provide a definite answer; they would extend, however, our study beyond its present scope.

**Conclusions.** The observation that histamine per se is able to produce an increase in eosinophils in the blood of sensitized guinea pigs, might explain Campbell’s findings that it enhances the eosinophilic response which follows the reinjection of homologous antigen. We hesitate on the other hand to draw any more far-reaching conclusions as to the underlying mechanism, since Campbell reports even a larger increase by the use, in a corresponding technic, of acetylcholine and cysteine.

**VII. Correlation of Eosinophils in Bone Marrow, Peripheral Circulation and Shock Tissue**

**Experimental problem.** It had been recognized and stated by early investigators that any attempt to understand the mechanism of peripheral eosinophilia requires the simultaneous study of bone marrow, peripheral circulation and shock tissue. Curiously enough, no accord has been reached about the interpretation of findings. Weinberg and Seguin, for instance, found in a study of eosinophils in the blood and lungs of guinea pigs that 4 out of 10 animals sensitized but not reinjected with horse serum as well as 9 out of 22 animals sensitized and reinjected with horse serum had eosinophils in their lungs provided they had a high eosinophil count in their blood. They concluded that the presence of eosinophils in the lungs had no relation to anaphylaxis and termed the phenomenon chronic spontaneous eosinophilia.

Hajos, on the other hand, using the same species and the same antigen, based his conclusions on the examination of bone marrow, peripheral blood count and lungs of guinea pigs sensitive to horse serum prior to reinjection and from eight to forty-eight hours after reintroduction of the specific antigen by intramuscular injection or by inhalation. He found that of the three groups examined only those which were re-exposed to the antigen by inhalation showed a pulmonary eosinophilia. Homma, who used white rats injected with parasites and parasitic material, felt confident that he had established a direct relationship between eosinophils in bone marrow, blood and shock tissue. He maintained that eosinophils increase in the bone marrow during sensitization and, furthermore, that he had been able to correlate their subsequent decrease in the bone marrow with their increase in the peripheral circulation and their final decrease in the peripheral circulation with their increase in the shock tissue. The cycle thus established would confirm Ehrlich’s concept of the origin and distribution of eosinophils. Unfortunately, his paper contains neither figures nor a description of his methods, and the Japanese journal to which he refers for the details of his experimental procedure is not available to us. It is therefore impossible to reproduce his experiments. For this and other reasons mentioned earlier, we decided to study bone marrow and shock tissues of a series of guinea pigs which were sensitized and reinjected with horse serum by the standard procedure employed throughout our investigation.
The study of bone marrow presented us with a number of technical problems. Comparative studies convinced us that of the three available methods, marrow puncture, touch preparation of marrow, and marrow section, the last gave the most consistent results. The number of eosinophils within the area of a grid was the technic adopted.*

The examination of shock tissues does not offer any particular difficulties. The lungs which represent the essential shock tissue in the guinea pig were classified into six groups according to the number of eosinophils observed:

- no eosinophils: 0
- few eosinophils: (one or two per h.p.f.): +
- moderate eosinophilia: ++
- marked eosinophilia: +++
- massive eosinophilia: ++++

This corresponds roughly to the classification used by Weinberg and Seguin.17

* We are indebted to Dr. L. R. Limarzi for his suggestions and his help in identifying the preparations.
The eosinophils are seen to accumulate in the perivascular and peribronchial connective tissue. They are prevalent in septal tissue and bronchial walls and are imbedded in mucus in the lumen of the bronchi. As a rule, a large number surround the intrapulmonary lymphatic tissue which occurs normally in guinea pigs, and while a few are observed in lymphatic channels, the major portion is found in the periphery of the lymphnodes (fig. 1), a relationship which had been described by Opie15 more than forty years ago.

Experimental procedure. Guinea pigs (18) were sensitized to horse serum by the method previously described. Twenty-one days later, they were divided into three group of 6 animals each. One group was given 0.75 horse serum intracardially without protection; the second group received benadryl, 5 mg./Kg. in aqueous solution; the third group SY 18, 1.5 mg./Kg. in aqueous solution intraperitoneally, thirty minutes prior to the intracardial reinjection of horse serum. Three of the unprotected animals and two of the animals protected with SY 18 died from the result of immediate or delayed anaphylactic reactions.

Another series of guinea pigs (6) was sensitized by intraperitoneal injection of 0.75 cc. of 1 per cent ovomucin in aqueous solution. Twenty-one days later, the guinea pigs were divided into three groups of two animals each. One group was given 0.75 cc. of a 1 per cent aqueous solution of ovomucin intracardially without protection; the second group received benadryl, 5 mg./Kg. in aqueous solution; the third group SY 18, 1.5 mg./Kg. in aqueous solution, thirty minutes prior to the reinjection of ovomucin. One of the unprotected animals died from the results of immediate anaphylactic shock.

None of the animals had more than 1 per cent eosinophils prior to reinjection. Twenty hours after the intracardial reinjection, differential counts were obtained in all the animals, and in four nonsensitized guinea pigs. After the counts had been taken, the animals were sacrificed. The first animals were sacrificed by fracturing their necks. In view of the marked extravasation of blood which results from violent death, this procedure was abandoned and the majority of the animals were sacrificed by intracardial injection of nembutal in aqueous solution. Immediately after death, postmortem examinations were performed; specimens were removed of lungs and of any organ which showed gross pathologic changes. One femur was carefully opened, the bone marrow "penciled out" in toto. The specimens were fixed in Zenker-Formol, freshly prepared. They were imbedded in paraffin and stained with hematoxylin-azure-eosin. This stain permits a satisfactory identification of eosinophils in all preparations. A grid inserted into the ocular of the microscope was used for the counting of eosinophils in the bone marrow. The figures thus obtained are relative figures and not comparable with those quoted by other authors.

Results. The correlation of eosinophils in bone marrow, peripheral circulation and lungs is summarized in table 6.

1. Bone marrow: Eosinophil counts in the bone marrow of non-sensitized guinea pigs varied from 8 to 52 in the area of the grid used during this study; in the bone marrow of guinea pigs sensitized and reinjected with horse serum, from 7 to 62; in the bone marrow of guinea pigs sensitized and reinjected with ovomucin, from
### Table 5.—Percentage of eosinophils (before, three hours and twenty hours after reinjection) in guinea pigs sensitized and reinjected with horse serum

The first group had 1% or less, the second group from 2% to 7% eosinophils prior to reinjection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>% of Eosinophils</th>
<th>% of Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>12 guinea pigs without eosinophils prior to reinjection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>12 guinea pigs with eosinophils present prior to reinjection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% before reinjection</th>
<th>% 3 hrs. after reinjection</th>
<th>% 24 hours after reinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
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</tr>
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<tr>
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<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% before reinjection</th>
<th>% 3 hrs. after reinjection</th>
<th>% 20 hours after reinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
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<td>9</td>
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<tr>
<td>2</td>
<td>3</td>
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</tr>
<tr>
<td>7</td>
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<tr>
<td>6</td>
<td>7</td>
<td>14</td>
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### Table 6.—Correlation between eosinophils in bone marrow, peripheral blood and shock tissue, in normal controls and guinea pigs sensitized and reinjected with horse serum and ovomucin, twenty hours after reinjection

<table>
<thead>
<tr>
<th>No.</th>
<th>Antigen</th>
<th>Symptoms</th>
<th>Eosinophils</th>
<th>Anti-histamine drug used</th>
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<td>Horse serum</td>
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<td>19</td>
<td>2</td>
</tr>
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<td>52</td>
<td>0</td>
</tr>
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<td>Horse serum</td>
<td>+</td>
<td>62</td>
<td>17</td>
</tr>
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<td>24</td>
<td>7</td>
</tr>
<tr>
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<td>29</td>
<td>1</td>
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<td>6</td>
</tr>
<tr>
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<td>Horse serum</td>
<td>±</td>
<td>22</td>
<td>12</td>
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<tr>
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<td>0</td>
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<td>1</td>
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<tr>
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<td>15</td>
<td>19</td>
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<tr>
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<td>12</td>
<td>5</td>
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<td>10</td>
<td>0</td>
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</table>
The average of the animals reinjected with horse serum is slightly higher, and of those reinjected with ovomucin considerably higher, than the average of nonsensitized controls. We believe that the difference between nonsensitized and sensitized animals is likely to be more pronounced before rather than after the reinjection which causes a redistribution of eosinophils. Two animals must be classified as experimental failures: guinea pig No. 325 failed to respond to sensitization and reinjection with horse serum; it showed for unexplained reasons neither anaphylactic symptoms nor tissue reactions. Guinea pig No. 335 is listed without its bone marrow count since a technical mishap made the specimen unfit for interpretation.

The variation between the eosinophil counts in the bone marrow of individual guinea pigs is so marked that the examination of the bone marrow does not permit any conclusion as to whether it has been obtained from a non-sensitized animal or from an animal which has been sensitized and reinjected with the specific antigen. We have not been able to establish any correlation between the number of eosino-
phils in bone marrow and peripheral blood. Five guinea pigs which had peripheral counts of 10, 12, 16, 17 and 19 per cent respectively, had, in the corresponding order, bone marrow counts of 17, 22, 29, 15 and 62 eosinophils in the grid area.

2. Lungs: Of the nonsensitized controls, eosinophils were absent in 2 specimens, one showed a few eosinophils, one a moderate number and none of the controls had eosinophils in their peripheral blood at the time of death. Of the guinea pigs sensitized and reinjected with horse serum, 3 failed to show eosinophils in the lungs; 4 showed a few eosinophils; 4 had a moderate, 1 a marked and 1 a massive eosinophilia. Of the guinea pigs sensitized and reinjected with ovomucin, 2 failed to show eosinophils, 2 showed a few eosinophils, 1 had a marked eosinophilia. Of the 5 animals which showed a moderate eosinophilia in their lungs, 3 had a peripher al eosinophil count of 5 per cent or more; the animals (3) which had a marked or massive pulmonary eosinophilia, had peripheral eosinophil counts of 12, 16 and 17 per cent. With the exception of the control (guinea pig No. 416), the animals which displayed a moderate pulmonary eosinophilia, had been protected with benadryl, those which showed a marked or massive eosinophilia, with SY 18. On the other hand, eosinophils were absent in one of the animals treated with benadryl, one of the animals treated with SY 18; a few eosinophils were seen in the remaining animals of either group. Anti-histamine drugs did not abolish the eosinophilic response in the lungs of guinea pigs sensitized and reinjected with antigen. Figure 2 shows the histologic section of the lung of guinea pig No. 332 protected by benadryl.

A definite correlation appears to exist between peripheral eosinophilia and shock tissue. All of the animals which had more than 10 per cent eosinophils in the peripheral blood, showed a moderate, marked, or massive number of eosinophils in their lungs.

Discussion. 1. Bone marrow: Hajos who tried to correlate eosinophilia in bone marrow, blood and lungs, found percentages of 1.3 per cent and 1.5 per cent of cells with eosinophilic granules—myelocytes, myeloblasts and mature cells—in the bone marrow of normal guinea pigs. He counted a total of 1000 bone marrow cells—a technic comparable to our own. Accepting these figures as a base line, he proceeded to examine the bone marrow of guinea pigs sensitized to horse serum. The percentage of eosinophils increased to a maximum of 9 per cent, while the peripheral eosinophil count failed to rise. Intramuscular reinjection of the specific antigen decreased the percentage of eosinophils slightly, inhalation of the specific antigen considerably. The differential count of the bone marrow, eight to twenty-four hours after exposure of the sensitized guinea pig to nebulized horse serum, ranged from 1 per cent to 3 per cent. At the time of the decrease in eosinophils of the bone marrow, a slight increase was noted in the peripheral blood; it did not exceed, however, 4 per cent in the latter group. We have been unable to confirm the existence of a "normal" eosinophil count in the bone marrow of guinea pigs.

The pronounced variation, in our opinion, is explained by the actual difference in the number of eosinophils present in the bone marrow of each individual animal, but is enhanced by the characteristics of distribution of eosinophilic cells within the same bone marrow. Figure 3, a bone marrow section of guinea pig No. 411,
illustrates the irregularity of the pattern which becomes even more significant when the percentage of eosinophils is low. The latter consideration is of minor importance: examination of several sections and increase of the total number of cells counted might establish a valid average. While it might thus be possible to observe increase or decrease of cells with eosinophilic granulation in the same animal, we do not believe that changes in the percentage of eosinophils in the bone marrow of different guinea pigs are comparable.

2. Lungs: The study of eosinophils in shock tissues has assumed renewed importance, since Halpern\textsuperscript{49} reported that the injection of antihistamine drugs derived from thiodiphenylamine prevented the eosinophilia usually found in the lungs of guinea pigs twenty-four hours after the anaphylactic reaction. Halpern, in his experiments, used sheep serum as antigen; the sensitizing dose was given intra-peritoneally in two portions, three days apart; the shocking dose, fifteen to twenty-one days later into the jugular vein. He states that this technic causes fatal shock in 100 per cent of unprotected animals. Its anaphylactic antigenicity appears to be superior to the horse serum used in our experiments; we have no information about the peripheral eosinophilic response in the animals thus sensitized. If the series
of antihistamine drugs studied by Halpern prevents the development of an eosinophilia in shock tissues, while the type of preparations which we have used either fails to influence or even increases the number of eosinophils present, the difference thus established should provide a clue of fundamental significance.

3. Correlations: The concept of "chronic spontaneous eosinophilia" which we have mentioned in the beginning of this section has been developed by Weinberg and Seguin as the result of comparative studies of blood and lungs in sensitized guinea pigs before and after reinjection of the specific antigen. We have no cause to doubt the validity of their findings, but we are certain that the observations upon which the concept is based require re-evaluation, because a large number of the experimental animals had a high peripheral eosinophil count prior to reinjection. The importance of this distinction becomes evident, if we analyse the factors which might influence the number of eosinophils in blood and shock tissue. We have reason to assume that a specific stimulus such as originates during the antigen antibody reaction is necessary to produce a peripheral eosinophilia. Opie, Weinberg and Seguin, and recently Ingraham and Wortman have demonstrated beyond doubt, however, that the chemotactic behavior of eosinophils equals that of neutrophils; that they are, moreover, phagocytic in vitro and in vivo. Accordingly, if they are once present in the blood stream, a variety of pathological changes might account for their presence in various tissues; this is one of the considerations which has caused us to restrict our studies to guinea pigs which had no peripheral eosinophilia prior to reinjection of the specific antigen.

The second factor which might influence the distribution of eosinophils in blood and shock tissue is even more pertinent because it applies to our own experiments in which the antigen antibody reaction was the immediate and undisputable cause for appearance of eosinophils in the circulation. Our studies seem to confirm Weinberg and Seguin's observations which correlate peripheral and pulmonary eosinophilia. Yet it has to be pointed out that this apparent correlation might be seriously distorted. Gerlach in his classic experiments had emphasized the importance of factors which determine where the antigen antibody reaction is localized. The lungs represent only one of the shock tissues of the guinea pig: the entire gastrointestinal tract, bladder, uterus, and skin are bound to participate in antigen antibody reactions and to influence the distribution of eosinophils. It is quite likely, as a matter of fact, that the total number of eosinophils present in shock tissues, other than lungs, might be considerably larger than the pulmonary fraction. Figure 4 represents a typical example: a section of the stomach of guinea pig No. 323 which was sensitized and reinjected with horse serum. The guinea pig, although not protected by antihistamine drugs, did not exhibit anaphylactic symptoms; it was sacrificed twenty hours after reinjection. It had a peripheral eosinophil count of 7 per cent at the time of its death. Only few eosinophils are seen in its lungs; the stomach, on the other hand, shows marked edema and a massive eosinophilia. It is obvious that in the representative case of the animal which we have just described, a comparative study which disregards the gastric reaction would be of doubtful value. We were impressed by the apparent affinity of eosinophils to the connective tissue of the specimens which we have examined.
It seems conceivable to us that the eosinophilic response might require the interaction of a factor which originates during the antigen antibody reaction with a connective tissue component present in shock organs. We have no concept of the particular manner in which the connective tissue might participate in the process of sensitization, but we suspect that further studies of the function of eosinophils will also uncover a specific function of the connective tissue which has, so far, escaped our attention.

While we feel that the results of our studies on correlation are of interest with regard to the action of antihistamine drugs, we are also aware of the fact that the actual problem of the mechanism which correlates the presence of eosinophils in blood and lungs has not been solved. It is not possible to decide by "static" in-
vestigations such as ours and those of previous workers whether the increase of eosinophils in the blood is the cause or the result of the eosinophilia observed in shock tissue. It might be necessary to study the eosinophilic response in shock organs which are isolated in vivo and permit the continuous determination of the percentage of eosinophils in the arterial and venous circulation with which they are supplied. In humans, the investigation of the eosinophilic response in shock tissues has been confined to the skin.

Kline, Cohen and Rudolph found a marked eosinophilic infiltration in the skin of allergic individuals, twenty to twenty-five minutes after injection of either histamine or specific antigen. The initial count of 50 per cent decreased to about 10 per cent after three hours. Nonallergic persons failed to exhibit this transitory local eosinophilia; the injection of histamine caused only a slight inflammatory reaction.

Jadassohn, on the other hand, described local eosinophilia in the human skin after mechanical irritation, injection of morpbin, atropine, or pilocarpine, even in the absence of blood eosinophilia.

Knott and Pearson demonstrated in similar studies that the site of a positive skin test in allergic individuals contains, twenty minutes after injection of the antigen, approximately twice as many eosinophils as the peripheral blood; the site of a negative skin test in allergic individuals as well as in normal controls, a number which equals the peripheral count. The injection of histamine causes a local eosinophilia in the wheals formed in either nonallergic or allergic individuals; it is twice as high as the peripheral count in the former, two and one-half times as high in the latter.

It must be assumed that the discrepancies in the findings of the three authors are due to a lack of uniformity in those factors which determine genesis and extent of local eosinophilia, e.g., in type and degree of individual sensitivity. We hope that the controlled conditions of the animal experiment will permit us to arrive at conclusions which clarify the open question about the agents responsible for the presence of eosinophils in shock tissues.

VIII. Conclusions

Our results appear to have clarified a number of questions which have obscured the investigation of the eosinophilic response. It has been shown that discrepancies found in the literature are largely due to variations inherent in the nature of the antigen, its route of administration and variations in the responsiveness of the experimental animal. We have standardized our experimental procedure to eliminate as many variables as possible. "Shock" per se does not seem to account for the eosinophilia which develops subsequent to the reinjection of the specific antigen in sensitized guinea pigs. The eosinophilic response, unlike the anaphylactic reaction, is not abolished by the antihistamine drugs which we have used. Observation of eosinophils in the blood and tissue of animals thus protected suggest that there might be important differences between the various types of antihistamine drugs which are now available. We have, finally, analyzed the possible correlation between eosinophilia in bone marrow, peripheral circulation and shock tissue. Although such correlation has been found to exist in several instances, we have also come to realize the limitations which prohibit far-reaching conclusions.

It might be permissible to discuss briefly possible avenues of future approach. In view of the fact that eosinophils appear subsequent to sensitization and reinjection of antigen, several investigators have attempted to relate the function of the
Weinberg and Seguin, for instance, sensitized guinea pigs with repeated intraperitoneal injections of hydatid fluid and obtained a peritoneal exudate rich in eosinophils. The cells were washed, counted, suspended in a measured amount of hydatid fluid and incubated; complement fixation tests on the fluid before and after incubation revealed a loss of antigen proportional to the number of eosinophils in the exudate. The experimental technic used by these authors, however, permits interpretations other than those proffered; we hesitate to accept their conclusion that the antigen has been absorbed by the eosinophils which, they suggest, might produce specific antibodies after absorption.

Three recent publications refer to in vitro experiments on the function of eosinophils in the mechanism of antigen antibody reactions. Ringoen writes: "Olson's recent studies of the eosinophils in immune reactions, indicate that a specific sensitizing product is formed between eosinophile leukocyte granules and complex proteins." Osgood, with Perlman, studied the development of eosinophils in bone marrow cultures. Eosinophils formed when the specific allergen was added to cultures of the marrow of allergic patients. They did not develop in cultures of bone marrow of nonallergic individuals which had been "sensitized" by addition of a small amount of allergic serum. Histamine did not produce eosinophilia in either allergic or nonallergic cultures. Kirk, finally, reports: "Dr. Houghton, in tissue cultures of cells from normal adults plus the serum of sensitive individuals, produced an increasing number of developing eosinophils; likewise the juvenile and adult eosinophile cells lived longer." In an attempt to secure additional details, we have communicated with each of the authors; unfortunately — in part due to circumstances beyond control — none has completed the work beyond this suggestive stage.

While it is conceivable that in vitro studies might result in the sudden discovery of the function of the eosinophil, in vivo experiments will accomplish the same objective by a steady process of elimination and change. We believe as Campbell does that whatever the function of the eosinophil may be it is "the same under all conditions." Of several theories, however, which have been advanced to explain the presence of eosinophils in blood and shock tissues after antigen antibody reactions, none has been confirmed.

IX. Summary

1. The eosinophilic response of the guinea pig sensitized and reinjected with the specific antigen varies with the nature of the antigen used, but also with the individual guinea pig in any groupsensitized and reinjected with the same antigen.
2. Certain antihistamine drugs which abolish anaphylactic symptoms, do not abolish the eosinophilic response.
3. The severity of anaphylactic "shock" symptoms has no influence on the eosinophilic response.
4. Histamine phosphate has no effect on the eosinophil count of nonsensitized guinea pigs protected by benadryl; it causes a distinct eosinophilic response in sensitized animals.
5. Heparin — in the dose injected — produced only an insignificant rise in the peripheral eosinophil count of sensitized guinea pigs; adenosine had no effect.
6. Attempts were made to correlate the eosinophilic response in bone marrow, blood and shock tissue of guinea pigs sensitized and reinjected with a specific antigen. The variation within a wide range of the number of eosinophils in the bone marrow of nonsensitized and of sensitized, reinjected guinea pigs is emphasized. A definite correlation seems to exist between the presence of a large number of
RESPONSE OF EOSINOPHILS IN GUINEA PIG

eosinophils in blood and lungs; it is shown, however, that this observation permits only limited conclusions.

7. The factors which account for discrepancies in the interpretation of the eosinophilic response, e.g., nature of antigen, route of administration and characteristics of species, are analyzed.

8. The significance of the findings is reviewed in the light of previous work.

ACKNOWLEDGMENT

We wish to express our appreciation to Miss Alice Sprenger for her patient and efficient technical assistance.

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RESPONSE OF EOSINOPHILS IN GUINEA PIG

MAX SAMTER

THE RESPONSE OF EOSINOPHILS IN THE GUINEA PIG TO SENSITIZATION, ANAPHYLAXIS AND VARIOUS DRUGS