A Comparative Study of the Blood of Certain Reptiles

by

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>4</td>
</tr>
<tr>
<td>Experimental Results</td>
<td>9</td>
</tr>
<tr>
<td>Cell Numbers, Erythrocyte Measurements, and Hemoglobin Content</td>
<td>9</td>
</tr>
<tr>
<td>Differential Leucocyte Counts</td>
<td>23</td>
</tr>
<tr>
<td>Sizes of Leucocytes</td>
<td>29</td>
</tr>
<tr>
<td>Cell Descriptions</td>
<td>32</td>
</tr>
<tr>
<td>Parasitism</td>
<td>37</td>
</tr>
<tr>
<td>Discussion</td>
<td>39</td>
</tr>
<tr>
<td>Summary and Conclusions</td>
<td>53</td>
</tr>
<tr>
<td>Bibliography</td>
<td>55</td>
</tr>
</tbody>
</table>
A Comparative Study of the Blood of Certain Reptiles

INTRODUCTION

The study of the blood and blood-forming tissues of infra-mammalian vertebrates has received more attention during the past few years than for some time previous. This renewed interest was apparently the result of a desire for new data to support various theories of hemopoiesis. Of the poikilothermic vertebrates, the Reptilia represent a higher development phylogenetically than the Amphibia, but more work has been done on the hematology of the latter.

The blood of only two species of reptiles common to this semi-arid region of southern Arizona has been studied heretofore. Edwards and Dill (1935) made a study of the physico-chemical properties of reptilian blood and reported, among other miscellaneous data, erythrocyte numbers and hemoglobin content of the blood of the Gila monster, *Heloderma suspectum*. Jordan and Speidel (1929) reported on the hemopoietic activity of the spleen and bone marrow of the horned toad, *Phrynosoma solare*, but did not give data on the types or numbers of cells present in the circulating blood.
The blood of several other species of reptiles has been used for various studies by other workers, both in this country and in Europe. Alder and Huber (1923) made a fairly complete morphological study of the blood of the following reptiles: *Lacerta muralis*, *Lacerta agilis*, *Anguis fragilis*, *Tarentola mauritanica*, and *Emys orbicularis*. Baker and Kline (1931) made erythrocyte enumerations for a number of representative vertebrates including two species of snakes and one species of turtle.

In their chemical studies of reptilian blood, Dill and Edwards gave erythrocyte numbers of the crocodile, *Crocodileus acutus* (1931); Dill, Edwards, Bock, and Talbott (1935) for the chuckwalla, *Sauromalus obesus*; and Dill and Edwards (1935) for the alligator, *Alligator mississippiensis*. Hopping (1923), in a chemical study of the blood of the alligator (Alligator species?), reported erythrocyte numbers which were somewhat higher than those given by Dill and Edwards. Weigmann (1932) found greater numbers of erythrocytes per cubic millimeter of blood in the winter period than for the rest of the year in the Old World lizard, *Lacerta vivipara* Jacq. Sabrazes and Muratet (1924 b) reported on the leucocytes of the blood of *Lacerta muralis*, and found a great deal of individual variation in regard to cell types and percentages present.

After considerable work on various reptiles, von Werzberg (1911) concluded that there were no typical neutrophiles
present in the blood. He did describe, however, mononuclear lymphoid cells with azurophilic granules which, in certain species, possessed indented nuclei. With this view Loewenthal (1930)(1931) did not concur, having found "neutrophiles veritables" in fish, amphibians, and reptiles, but not in birds.

Because of the incompleteness of the data on the hematology of reptiles as a whole, and in particular those of the semi-arid region of the southwest, the present study was undertaken to establish normal hematological pictures for certain representative species.

Ten species of reptiles were used, which fall into five general groups: geckos, Gila monsters, horned toads, snakes, and swifts. Determinations were made of erythrocyte and leucocyte numbers per cubic millimeter and hemoglobin content per 100 cubic centimeters of blood. The various blood cells were measured and described, and differential counts of the leucocytes were recorded.

The writer wishes to express his appreciation to Dr. G. T. Caldwell, who suggested this problem, and under whose direction it has been carried out, for his helpful suggestions and advice.
MATERIALS AND METHODS

A total of 53 animals captured in the vicinity of Tucson, with the exception of two of the bull snakes (S-3 and S-4) which were brought from near Phoenix, was used in the studies. When the animals were kept for more than a few weeks they were fed; the snakes and Gila monsters were given raw hen's eggs, and the horned toads were placed near ant hills to feed. Swifts and geckos were not fed, but were killed a short time after being captured.

The reptiles used were as follows: nine geckos, Col- eonyx variegatus; nine Gila monsters, Heloderma suspectum; seventeen horned toads, Phrynosoma solare; eight snakes including three bull snakes, Pituophis sayi; one each of the Arizona king snake, Lampropeltis pyromelana; ribbon snake, Thamnophis proxima; ring-necked snake, Diadophis regalis; spotted night snake, Hypsiglena ochrorhynchos; and a garter snake, Thamnophis marcellus; ten swifts, including seven desert scaly swifts, Sceloporus magister; one gridiron or zebra-tailed swift, Callisaurus ventralis; and two small specimens of the genus Sceloporus.* For eleven of the fifty-three animals the data are incomplete in regard to erythrocyte and leucocyte enumerations and

*Identification of genera and species made by Dr. G. T. Vorhies.
hemoglobin content, and for eight animals the smears were unsatisfactory for accurate differential counts.

Heart blood was used exclusively as most representative of the circulating blood. The smaller animals (geckos, small swifts, and small snakes) were anaesthetized with ether, and the body cavity opened in order to draw blood directly from the heart. Although no incisions were necessary in the case of the larger animals, ether was used to maintain a standard technique. From each of these larger animals two, or more, samples of blood were obtained on different days. The blood was drawn into a 2 cubic centimeter syringe containing a small crystal of sodium citrate to retard coagulation. Citrate solution could not be used as this would dilute the blood. The largest gauge needle (up to #22) which could be inserted into the ventricle of the heart was used. The smaller needles were not satisfactory because the blood could not be drawn through them with sufficient rapidity to prevent coagulation.

Satisfactory smears could be made only on very clean slides, so various methods of cleaning the slides were tried. The most satisfactory was found to be that developed by Sabin (1923). In addition to a cleansing with acid cleaning solution, the slides were given a high polish with jeweler's rouge and a silk cloth. A simple test for determining the better surface of the slide consisted
of gently blowing the breath on the slide and noting the
evaporation of the film of moisture. From a well polished
surface the moisture evaporated evenly; imperfections
showed as spots or rings during evaporation.

A small drop of blood was placed on the polished
slide from the tip of the needle. The smear was made by
drawing the blood after the edge of a special slide cut
for this purpose. This slide was approximately three-
fourths as wide as the slides upon which the smears were
made. Thus the edges of the film were available for
study in permanent preparations, which was desirable as
many of the leucocytes were found near the edges.

With a dilute Wright's stain (Gruebler's) the smears
stained well in two to three minutes after fixation, and
permanent mounts were made in neutral balsam. From these
preparations cells were studied and leucocytes measured
with an ocular micrometer. The erythrocytes were mea-
sured in hanging drop preparations made by mixing a drop
of blood with a drop of 0.7% sodium chloride solution con-
taining neutral red dye. The drop was then suspended
from a cover glass over the chamber of a depression
chamber slide. The cover glass was held in place and
sealed with a vaseline-paraffin mixture. The cells were
also studied in supravitaly stained preparations accord-
ing to the method of Sabin (1923) as modified by Scott
(1928). The vital dyes employed were neutral red, and a
mixture of neutral red and Janus green.

The enumerations of the erythrocytes and leucocytes were made with a standard hemocytometer with a Levy counting chamber (improved Neubauer ruling). Two 1-100 dilution pipettes were used to make duplicate counts for each sample of blood. Six counts were made from each pipette, and eighty small squares of the counting chamber were counted in each instance. The diluting fluid, made by adding a small amount of neutral red dye to a few cubic centimeters of 0.7% sodium chloride stock solution, was freshly prepared and filtered before use. Keyes (1929), in his work on the blood of birds, recommended that a strong fixative, such as 2% osmic acid, be used for a diluting fluid so that the nuclei of hemolyzed erythrocytes would not be included in the leucocyte count. The saline diluent has been found satisfactory and the dye made it possible to distinguish clearly between erythrocytes and leucocytes, and to count them from the same drop of diluted blood.

Blood was drawn directly from the tip of the needle into the diluting pipettes. After proper dilution (1-100), the pipettes were sealed with a rubber band and immediately placed on a rotating device as designed by Bryan and Garrey (1934). The pipettes were rotated for at least ten minutes before counting to insure uniform dispersion of the cells. If the cell enumerations were not made
within two hours after the blood was drawn, a clumping of the thrombocytes resulted in inaccuracies.

The hemoglobin content was determined with a Bausch and Lomb Hemoglobinometer (improved Newcomer model) according to the Newcomer method. The blood was taken directly from the tip of the needle (immediately after the making of the smears) and diluted in the pipette with one per cent hydrochloric acid. The solution was allowed to stand for at least thirty minutes before reading. The hemoglobinometer was clamped to a table in good light. After the first reading the prisms were dried and the cups interchanged for a second reading which was averaged with the first.

Since the presence of parasites might alter the blood pictures, the animals were autopsied and examined for parasites. For the parasitized animals the types of parasites, their location in the body, and the degree of infestation were recorded.
EXPERIMENTAL RESULTS

Cell Numbers; Erythrocyte Measurements, and Hemoglobin Content

The records of the individual animals, as shown in Table I, include experimental data as follows: dates of bleedings, numbers of erythrocytes and leucocytes per cubic millimeter of blood, average ranges in size of erythrocytes, and hemoglobin content in grams per 100 cubic centimeters of blood. Because of the small amount of blood in the geckos, these animals could be bled only once. For these animals, only one determination of hemoglobin is given; the estimate of 7 grams per 100 cubic centimeters for the group average was made from this complete and several partial determinations. Although most of the swifts were bled a second time, it will be seen that only two (L-4 and L-10) gave comparable results on a second bleeding. A great deal of individual variation is evidenced by these tables, especially in the numbers of erythrocytes and leucocytes per unit volume of blood.
## TABLE I

Cell Numbers, Erythrocyte Measurements, and Hemoglobin Content

<table>
<thead>
<tr>
<th>Animal</th>
<th>Erythrocytes (per cu. mm.)</th>
<th>Average Range in Size of Erythrocytes (in micra)</th>
<th>Hemoglobin (in gms.)</th>
<th>Leucocytes (per 100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geckos, Coleonyx variegatus</td>
<td></td>
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</tr>
<tr>
<td>G-5</td>
<td>12-27-35 418,000</td>
<td>9.1-11.6 x 18.2-21.5</td>
<td>19,000</td>
<td></td>
</tr>
<tr>
<td>G-6</td>
<td>2-1-36    407,000</td>
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<tr>
<td>G-7</td>
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<td>G-8</td>
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<td>G-9</td>
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<td>Hemoglobin (in gms. per 100 cc.)</td>
<td>Leucocytes (per cu. mm.)</td>
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<td><strong>Gila Monsters, Heloderma suspectum</strong></td>
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<td>GM-1</td>
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### TABLE I (continued)

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<tr>
<th>Animal</th>
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<th>Hemoglobin (in gms. per 100 cc.)</th>
<th>Leucocytes (per cu. mm.)</th>
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<td>T-16</td>
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Horned Toads, Phrynosoma solare
TABLE I (continued)

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<thead>
<tr>
<th>Animal</th>
<th>Erythrocytes (per cu. mm.)</th>
<th>Average Range in Size of Erythrocytes (in micra)</th>
<th>Hemoglobin (in gms. per 100 cc.)</th>
<th>Leucocytes (per cu. mm.)</th>
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<td><strong>Snakes</strong></td>
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<td>S-1 Lampropeltis pyromelana 12-1-34</td>
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<td>7.6--8.4 x 14.5-16.0</td>
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<td>S-2 Thamnophis marcianus 3-9-35</td>
<td>774,000</td>
<td>8.4-15.1 x 16.8-20.1</td>
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<td>6-6-35</td>
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TABLE I (continued)

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<th>Animal</th>
<th>Erythrocytes (per cu. mm.)</th>
<th>Average Range in Size of Erythrocytes (in micra)</th>
<th>Hemoglobin (gms. per 100 cc.)</th>
<th>Leucocytes (per cu. mm.)</th>
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<td>L-1 Sceloporus sp.</td>
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<td>11-25-34</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-15-35</td>
<td>1,366,000</td>
<td>8.4-12.6 x 14.3-18.5</td>
<td>13.1</td>
<td>80,600</td>
</tr>
<tr>
<td>4-26-35</td>
<td>745,000</td>
<td></td>
<td>7.8</td>
<td>58,750</td>
</tr>
<tr>
<td>L-4 Sceloporus magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-1-35</td>
<td>2,074,000</td>
<td>8.3-10.8 x 16.7-20.8</td>
<td>13.0</td>
<td>39,000</td>
</tr>
<tr>
<td>6-3-35</td>
<td>1,926,000</td>
<td></td>
<td>10.3</td>
<td>55,580</td>
</tr>
<tr>
<td>L-9 Sceloporus magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-23-35</td>
<td>1,045,000</td>
<td>9.1-11.4 x 16.0-19.4</td>
<td>9.8</td>
<td>43,750</td>
</tr>
<tr>
<td>4-1-36</td>
<td>771,250</td>
<td></td>
<td>6.7</td>
<td>27,500</td>
</tr>
<tr>
<td>L-10 Sceloporus magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-1-36</td>
<td>1,202,500</td>
<td>10.2-13.7 x 17.1-20.5</td>
<td>12.0</td>
<td>45,000</td>
</tr>
<tr>
<td>4-4-36</td>
<td>980,000</td>
<td></td>
<td>10.3</td>
<td>27,500</td>
</tr>
<tr>
<td>L-11 Sceloporus magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-1-36</td>
<td>849,150</td>
<td>10.2-12.5 x 17.1-20.5</td>
<td>7.2</td>
<td>36,250</td>
</tr>
<tr>
<td>4-4-36</td>
<td>629,000</td>
<td></td>
<td>6.6</td>
<td>28,750</td>
</tr>
<tr>
<td>L-12 Sceloporus magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-14-36</td>
<td>810,000</td>
<td>10.2-13.7 x 17.1-21.6</td>
<td>10.5</td>
<td>30,000</td>
</tr>
</tbody>
</table>
The experimental data of Table I are consolidated in Table II which gives the averages of the separate determinations made on each animal (except for the swifts), and also the averages for each group. After the withdrawal of the first sample of blood the swifts apparently had too little blood to be considered normal. Therefore the first determination is given as more nearly normal than the averages of the separate determinations.

### TABLE II

**Individual and Group Averages of Cell Numbers, Erythrocyte Measurements, and Hemoglobin Content**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Erythrocytes (per cu. mm.)</th>
<th>Average Range in Size of Erythrocytes (in micra)</th>
<th>Hemoglobin (in gms. per 100 cc.)</th>
<th>Leucocytes (per cu. mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-5</td>
<td>418,000</td>
<td>9.1-11.6 x 18.2-21.5</td>
<td></td>
<td>19,000</td>
</tr>
<tr>
<td>G-6</td>
<td>407,000</td>
<td>10.0-11.5 x 18.0-21.0</td>
<td></td>
<td>15,000</td>
</tr>
<tr>
<td>G-7</td>
<td>9.1-12.0 x 18.0-20.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-8</td>
<td>700,000</td>
<td>10.2-13.7 x 17.1-21.5</td>
<td>6.7</td>
<td>27,500</td>
</tr>
<tr>
<td>G-9</td>
<td>500,000</td>
<td>10.2-11.5 x 18.2-21.5</td>
<td></td>
<td>45,000</td>
</tr>
<tr>
<td>G-11</td>
<td>10.2-13.7 x 18.2-21.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-12</td>
<td>430,000</td>
<td>10.2-13.7 x 18.2-21.5</td>
<td></td>
<td>21,700</td>
</tr>
<tr>
<td>Average</td>
<td>491,000</td>
<td>10.0-12.5 x 18.0-21.2</td>
<td>7.0*</td>
<td>25,640</td>
</tr>
</tbody>
</table>

*Estimated from one complete and several partial determinations.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Erythrocytes (per cu. mm.)</th>
<th>Average Range in Size of Erythrocytes (in micra)</th>
<th>Hemoglobin (in gms. per 100 cc.)</th>
<th>Leukocytes (per cu. mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gila Monsters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-1</td>
<td>1,197,612</td>
<td>10.0-12.5 x 16.7-22.5</td>
<td>10.05</td>
<td>55,187</td>
</tr>
<tr>
<td>GM-2</td>
<td>959,500</td>
<td>10.8-14.1 x 17.5-22.5</td>
<td>10.65</td>
<td>32,832</td>
</tr>
<tr>
<td>GM-3</td>
<td>515,925</td>
<td>10.1-13.3 x 17.5-21.6</td>
<td>6.95</td>
<td>32,900</td>
</tr>
<tr>
<td>GM-4</td>
<td>802,500</td>
<td>10.9-12.6 x 16.8-21.0</td>
<td>7.10</td>
<td>25,850</td>
</tr>
<tr>
<td>GM-5</td>
<td>365,823</td>
<td>10.7-14.7 x 19.6-22.5</td>
<td>5.73</td>
<td>27,500</td>
</tr>
<tr>
<td>GM-6</td>
<td>472,083</td>
<td>12.7-14.7 x 17.6-22.5</td>
<td>6.45</td>
<td>26,287</td>
</tr>
<tr>
<td>GM-7</td>
<td>600,000</td>
<td>11.8-13.5 x 17.6-21.0</td>
<td>7.20</td>
<td>20,850</td>
</tr>
<tr>
<td>GM-8</td>
<td>325,800</td>
<td>10.2-13.7 x 17.1-22.8</td>
<td>4.50</td>
<td>21,250</td>
</tr>
<tr>
<td>GM-9</td>
<td>576,250</td>
<td>11.4-13.7 x 18.2-22.8</td>
<td>6.65</td>
<td>37,900</td>
</tr>
<tr>
<td>Average</td>
<td>646,278</td>
<td>10.8-13.6 x 17.7-22.2</td>
<td>7.25</td>
<td>31,173</td>
</tr>
<tr>
<td>Horned Toads</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-1</td>
<td>906,000</td>
<td>9.6-12.8 x 14.4-19.2</td>
<td>10.30</td>
<td></td>
</tr>
<tr>
<td>T-2</td>
<td>733,500</td>
<td>10.6-12.6 x 17.6-23.5</td>
<td>7.40</td>
<td>38,000</td>
</tr>
<tr>
<td>T-3</td>
<td>745,000</td>
<td>10.9-12.6 x 14.3-21.0</td>
<td>8.25</td>
<td>29,425</td>
</tr>
<tr>
<td>T-4</td>
<td>662,875</td>
<td>10.0-12.6 x 15.0-19.3</td>
<td>6.05</td>
<td>22,875</td>
</tr>
<tr>
<td>T-5</td>
<td>866,000</td>
<td>9.2-12.6 x 14.3-21.5</td>
<td>8.80</td>
<td>21,350</td>
</tr>
<tr>
<td>T-6</td>
<td>865,600</td>
<td>9.2-13.3 x 15.0-20.8</td>
<td>6.50</td>
<td>28,435</td>
</tr>
<tr>
<td>T-7</td>
<td>695,000</td>
<td>10.2-12.6 x 16.7-21.0</td>
<td>6.90</td>
<td>27,800</td>
</tr>
<tr>
<td>T-8</td>
<td>1,204,000</td>
<td>10.1-12.5 x 16.7-20.8</td>
<td>11.10</td>
<td>40,212</td>
</tr>
<tr>
<td>T-9</td>
<td>900,500</td>
<td>10.0-13.3 x 15.8-20.0</td>
<td>8.00</td>
<td>49,500</td>
</tr>
<tr>
<td>T-10</td>
<td>745,425</td>
<td>9.2-12.5 x 16.7-20.0</td>
<td>7.55</td>
<td>28,325</td>
</tr>
<tr>
<td>T-11</td>
<td>515,283</td>
<td>11.4-13.7 x 17.1-21.7</td>
<td>5.80</td>
<td>23,833</td>
</tr>
<tr>
<td>T-12</td>
<td>492,950</td>
<td>10.2-13.7 x 17.1-22.8</td>
<td>5.50</td>
<td>22,345</td>
</tr>
<tr>
<td>T-13</td>
<td>865,000</td>
<td>11.4-13.7 x 17.6-21.5</td>
<td>9.00</td>
<td>32,900</td>
</tr>
<tr>
<td>T-14</td>
<td>546,500</td>
<td>10.9-12.6 x 17.3-20.6</td>
<td>5.75</td>
<td>26,250</td>
</tr>
<tr>
<td>T-15</td>
<td>673,250</td>
<td>11.4-14.7 x 17.4-21.5</td>
<td>7.85</td>
<td>29,575</td>
</tr>
<tr>
<td>T-16</td>
<td>506,500</td>
<td>9.2-12.5 x 16.0-22.8</td>
<td>5.60</td>
<td>33,200</td>
</tr>
<tr>
<td>Average</td>
<td>744,654</td>
<td>10.2-13.0 x 16.2-21.0</td>
<td>7.52</td>
<td>30,280</td>
</tr>
<tr>
<td>Animal</td>
<td>Erythrocytes (per cu. mm.)</td>
<td>Average Range in Size of Erythrocytes (in micra)</td>
<td>Hemoglobin (in gms. per 100 cc.)</td>
<td>Leucocytes (per cu. mm.)</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Snakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1</td>
<td>670,000</td>
<td>7.6--8.4 x 14.3-16.0</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td>S-2</td>
<td>720,000</td>
<td>8.4-15.1 x 16.8-20.1</td>
<td>7.05</td>
<td>32,700</td>
</tr>
<tr>
<td>S-3</td>
<td>1,317,250</td>
<td>10.0-14.1 x 16.7-19.2</td>
<td>10.75</td>
<td>50,750</td>
</tr>
<tr>
<td>S-4</td>
<td>1,043,250</td>
<td>9.2-11.7 x 17.5-20.8</td>
<td>8.40</td>
<td>54,940</td>
</tr>
<tr>
<td>S-7</td>
<td>773,000</td>
<td>11.2-12.9 x 17.2-21.4</td>
<td>7.30</td>
<td>45,000</td>
</tr>
<tr>
<td>S-8</td>
<td>723,300</td>
<td>10.2-13.7 x 17.1-23.9</td>
<td>7.50</td>
<td>32,000</td>
</tr>
<tr>
<td>Average</td>
<td>874,470</td>
<td>9.6-12.6 x 16.1-20.2</td>
<td>8.50</td>
<td>42,877</td>
</tr>
<tr>
<td>Swifts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-1</td>
<td>2,110,000</td>
<td>11.1-16.7 x 12.9-17.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-2</td>
<td>540,000</td>
<td>7.9-15.2 x 15.0-17.5</td>
<td></td>
<td>18,000</td>
</tr>
<tr>
<td>L-3</td>
<td>1,366,000</td>
<td>8.4-12.6 x 14.3-18.5</td>
<td>13.10</td>
<td>80,600</td>
</tr>
<tr>
<td>L-4</td>
<td>2,074,000</td>
<td>8.3-10.8 x 16.7-20.9</td>
<td>13.00</td>
<td>47,265</td>
</tr>
<tr>
<td>L-9</td>
<td>1,045,500</td>
<td>9.1-11.4 x 16.0-19.4</td>
<td>9.80</td>
<td>43,750</td>
</tr>
<tr>
<td>L-10</td>
<td>1,202,500</td>
<td>10.2-13.7 x 17.1-20.5</td>
<td>12.00</td>
<td>45,000</td>
</tr>
<tr>
<td>L-11</td>
<td>849,150</td>
<td>10.2-12.5 x 17.1-20.5</td>
<td>7.20</td>
<td>36,250</td>
</tr>
<tr>
<td>L-12</td>
<td>810,000</td>
<td>10.2-13.7 x 15.7-19.5</td>
<td>10.50</td>
<td>30,000</td>
</tr>
<tr>
<td>Average</td>
<td>1,224,525</td>
<td>9.4-12.5 x 15.7-19.5</td>
<td>10.93</td>
<td>42,980</td>
</tr>
</tbody>
</table>

1First determinations that were made for each animal are given (from Table I).

2Not included in averages of erythrocyte numbers or hemoglobin content because data are incomplete.
In order to compare the results for the various groups, the averages from each group were combined in Table III. The groups, when arbitrarily listed in order of increasing hemoglobin content per unit volume of blood, showed an increase in each successive group in the numbers of erythrocytes per unit volume of blood. The sizes of the erythrocytes, as given, represent the means of the average ranges for the groups. It will be noted that there was a decrease in this mean size from the Gila monsters to the swifts. The mean size of the erythrocytes of the geckos was somewhat less than that of the Gila monsters, and slightly greater than that of the horned toads. Since these mean dimensions represent the width and length of the erythrocytes, it will be seen that the shape of the erythrocytes varied.
### TABLE III

Averages of Erythrocyte Numbers and Hemoglobin Content, and Mean Size of Erythrocytes for each Group

<table>
<thead>
<tr>
<th>Group (and number of individuals)</th>
<th>Erythrocytes (per cu. mm.)</th>
<th>Mean Size of Erythrocytes (in micra)</th>
<th>Hemoglobin (gms. per 100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geckos----5</td>
<td>491,000</td>
<td>11.25 x 19.60</td>
<td>7.00*</td>
</tr>
<tr>
<td>Gila Monsters--9</td>
<td>646,278</td>
<td>12.20 x 19.90</td>
<td>7.25</td>
</tr>
<tr>
<td>Horned Toads----16</td>
<td>744,650</td>
<td>11.60 x 18.60</td>
<td>7.50</td>
</tr>
<tr>
<td>Snakes----6</td>
<td>874,470</td>
<td>11.10 x 18.10</td>
<td>8.50</td>
</tr>
<tr>
<td>Swifts----6</td>
<td>1,240,330</td>
<td>10.90 x 17.40</td>
<td>10.73</td>
</tr>
</tbody>
</table>

*Estimated from one complete and several partial determinations.*
From a rather comprehensive survey of recent literature the following summary has been compiled, and is presented as a basis for comparisons with the data obtained in the present study.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Animal</th>
<th>Number of Animals</th>
<th>Numbers of Erythrocytes (per cu. mm.)</th>
<th>Size of Erythrocytes (in micra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alder and Huber (1923)</td>
<td><em>Lacerta agilis</em> (quite a few)</td>
<td>945,000</td>
<td>14.0-15.5 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lacerta muralis</em></td>
<td>1,447,000</td>
<td>12.5-14.0 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Anguis fragilis</em></td>
<td>1,516,000</td>
<td>8.5-10.0 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Tarentola mauritanica</em></td>
<td>602,000</td>
<td>15.5-18.5 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Emys orbicularis</em></td>
<td>503,000</td>
<td>15.5-18.5 x</td>
<td></td>
</tr>
<tr>
<td>Baker and Kline (1931)</td>
<td><em>Coluber constrictor</em></td>
<td>1</td>
<td>777,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Heterodon contortrix</em></td>
<td>1</td>
<td>603,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Terrapene carolina</em></td>
<td>1</td>
<td>619,000</td>
<td></td>
</tr>
<tr>
<td>Dill and Edwards (1931)</td>
<td><em>Crocodilus acutus</em></td>
<td>3</td>
<td>810,000</td>
<td></td>
</tr>
<tr>
<td>Dill et al. (1935)</td>
<td><em>Heloderma suspectum</em></td>
<td>8 (lot 1)</td>
<td>710,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (lot 2)</td>
<td>670,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edwards and Dill (1935)</td>
<td><em>Sauromalus obesus</em></td>
<td>8</td>
<td>910,000</td>
<td></td>
</tr>
<tr>
<td>Dill and Edwards (1935)</td>
<td><em>Alligator mississippiensis</em></td>
<td>7 (lot 1)</td>
<td>640,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 (lot 2)</td>
<td>490,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hopping (1923)</td>
<td><em>Alligator</em> (species?)</td>
<td>5</td>
<td>616,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>to 1,480,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weigmann (1932)</td>
<td><em>Lacerta vivipara</em></td>
<td>73</td>
<td>1,668,000 (winter)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,563,000 (summer)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The data concerning the average erythrocyte numbers and hemoglobin content per unit volume of blood, presented in Table III, are shown graphically in Figure 1. The groups of animals are arranged, as before, in order of increasing hemoglobin content. As previously noted, the numbers of erythrocytes also show an increase, but the increase in erythrocyte numbers is not directly proportional to the increase in hemoglobin content of the blood of the different groups. Comparing the end groups we see that the relations between erythrocyte numbers and hemoglobin content are reversed, the central groups showing intermediate stages.
Fig. 1 Comparative relations of erythrocytes and hemoglobin per unit volumes, in five groups of reptiles, arranged in order of increasing hemoglobin content.
Differential Leucocyte Counts

Table IV gives the differential leucocyte counts for the individual animals as made from the Wright's stained smears. A total of 1000 cells was examined for each animal except in a few cases in which the slides were not satisfactory for counting such a large number of cells. Considerable difficulty was experienced in accurately classifying many of the cells. Although Blain (1928) found it hard to distinguish between the free nuclei of disintegrated erythrocytes and the lymphocytes in the blood of birds, little difficulty was encountered on this point. The differentiation between small and medium sized lymphocytes was necessarily an arbitrary one, depending upon the relative amounts of cytoplasm present, because they represent different stages in the development of the same cell type. The distinction between monocytes and medium sized lymphocytes depended to a large extent on the sensitivity of the stain. The cytoplasm of the monocytes showed a slightly purple tinge in contrast to the clear blue of the cytoplasm of the lymphoid cells. The counts for certain indicated animals were not included in the averages because they showed definite abnormalities.
### TABLE IV
Differential Leucocyte Counts (in per cent)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Thrombocytes</th>
<th>Lymphocytes (small)</th>
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*Not included in Table V (group averages) because of abnormally large numbers of eosinophiles.
TABLE IV (continued)

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Snakes

| S-1    | 70.2                | 17.0               | 1.4                  | 7.4       | 2.9          | 1.1        |
| S-2    | 69.0                | 10.4               | 8.2                  | 5.1       | 7.0          | 0.3        |
| S-3    | 50.4                | 34.6               | 7.2                  | 4.1       | 3.7          |            |
| S-4    | 40.9                | 23.2               | 13.7                 | 9.4       | 2.8          |            |
| S-52   | 50.0                | 27.1               | 5.5                  | 7.1       | 9.6          | 0.7        |
| Thamnophis proxima | 29.2               | 1.2                | 1.3                  | 56.9      | 10.9         | 0.5        |
| S-6    | Diadophis regalis   | 62.2               | 21.7                 | 6.1       | 3.7          | 2.9        |
| S-8    | 61.5                | 16.9               | 6.0                  | 7.5       | 1.9          | 6.2        |

1 Not included in Table V (group averages) because of abnormally large numbers of eosinophiles.

2 Not included in Table V (group averages) because of abnormally large numbers of monocytes.
<table>
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<th>Animal</th>
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<th>Lymphocytes (medium)</th>
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*Not included in Table V (group averages) because of abnormally large numbers of basophiles.
Comparisons of the total and differential leucocyte counts may be made from Table V. Although the percentages of thrombocytes showed less variation than the percentages of any other cell type, little correlation is to be found either among cell types of a single group, or among cell types of the various groups. The most outstanding variations to be found in the percentages of cell types were the occurrence of a high percentage of basophiles in the geckos, and a low percentage of eosinophiles in the snakes.
<table>
<thead>
<tr>
<th>Group (and number of individuals)</th>
<th>Leucocytes (per cu. mm.)</th>
<th>Thrombocytes %</th>
<th>Lymphocytes (small) %</th>
<th>Lymphocytes (medium) %</th>
<th>Mono- cytes %</th>
<th>Eosinophiles %</th>
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Sizes of Leucocytes

Cell measurements, as shown in Table VI, represent the ranges in size of the leucocytes for the individual animals. These measurements were made with an ocular micrometer from the Wright's stained smears. The ranges were determined from the measurement of a large number of cells. Table VII gives the average ranges in size of the leucocytes for the groups.
### TABLE VI

**Individual Ranges of Leucocyte Sizes**

*in micra*

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<tr>
<th>Animal</th>
<th>Thrombocytes</th>
<th>Lymphocytes (small)</th>
<th>Lymphocytes (medium)</th>
<th>Monocytes</th>
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Swifts

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TABLE VII

Average Ranges of Leucocyte Sizes of the Groups
(in micra)

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<th>Lymphocytes (medium)</th>
<th>Monocytes</th>
<th>Eosinophiles</th>
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Cell Descriptions

Erythrocytes

The erythrocytes of all the animals studied had the same general appearance. They were ellipsoidal cells, with a nucleus about one third as large as the entire cell. The mean cell size of the erythrocytes of the various groups is given in Table III. In the supravital stains the few mitochondria were green, and the few small vacuoles were red. The cytoplasm did not stain but remained a light lemon yellow. When colored with Wright's stain, the nucleus was deeply basophilic and the chromatin appeared in irregular clumps. The cytoplasm varied from greenish-yellow to red, depending upon the intensity of the staining reaction and the concentration of the hemoglobin within the cytoplasm. The immature erythrocytes were easily recognized because of the relatively large vesicular nucleus, and the polychromatophilia of the cytoplasm which produced a greenish tinge with Wright's stain.

Thrombocytes

The thrombocytes were elongated fusiform or spindle shaped cells with relatively large nuclei. There was usually a longitudinal cleft or groove extending the length of the nucleus, which was described also by Jordan (1925) in the thrombocytes of the frog. In the suprav-
vital stains a few mitochondria were discerned and small vacuoles (3-12) were colored a reddish brown. As the preparation aged, the vacuoles increased in numbers and size, and the entire cell often became nearly spherical. The thrombocytes were often found in clumps or aggregations, appearing to form a syncytium. In the smears the thrombocytes were not definitely spindle shaped, but the shapes varied with the conditions under which the smears were made. The average range in size (Table VII) was approximately the same for all the groups studied. The cytoplasm was lightly basophilic while the nucleus, which often showed the longitudinal groove, was deeply basophilic with Wright's stain. (Plate I, Fig. 2; Plate II, Fig. 9).

Lymphocytes

The lymphocytes were the only cells found in reptilian blood which were morphologically indistinguishable from their counterparts of mammalian blood, although they were somewhat larger. The supravital stains made visible the mitochondria, and a comparatively large number (3-9) of reddish granules or vacuoles. The latter increased in size and numbers as the preparation aged. The nucleus of the smaller cells was relatively large and usually indent- ed or notched. The main mass of the scanty cytoplasm, in
which the mitochondria were found, was accumulated opposite the indentation. In the Wright's stained smears the nuclear chromatin was disposed in irregular angular blocks, with one or two nucleoli discernible. The cytoplasm was clear, and lightly basophilic. (Plate II, Figs. 10-11).

Monocytes

When treated with vital stains, the cytoplasm of the monocytes was seen to be more or less filled with lightly stained vacuoles, which became very large and deeply colored after several hours. The numerous scattered mitochondria were made evident with Janus green. A nucleolus was prominent within the hyaline or glass-like nucleus. The range in size of the monocytes was somewhat greater than that found in the lymphocytes (Table VII). With Wright's stained smears it was possible to differentiate between the lymphocytes and the monocytes because the nuclei of the latter were eccentric, and of a kidney or horse-shoe shape, (Plate I, Figs. 2-3). The bluish violet cytoplasm of the monocytes was also in distinct contrast to the clear blue of the lymphocytes, (Plate II, Figs. 10-12). In some of the animals there appeared cells very similar to the monocytes but with varying amounts of minute red granules in the cytoplasm. These granules were usually concentrated near the periphery, but were clumped
sometimes opposite the indentations of the nuclei, and occasionally distributed throughout the cytoplasm. The nuclei of these cells were often like those of the monocytes, but some were extremely polymorphic and simulated the appearance of the nuclei of mammalian neutrophiles. (Plate II, Figs. 13-14).

Eosinophiles

The eosinophiles of these reptiles were somewhat larger than those of man. In vital stains these cells were easily recognized because of the comparatively large granules which appeared to fill the cytoplasm. These stained strongly in neutral red and appeared as short rods or spindle shaped bodies of uniform size. After a time the granules often showed a radial arrangement, the peripheral ones were more or less rounded while those more centrally located were fusiform with their axes converging on a clear area near the center of the cell. Within a few hours red vacuoles appeared which increased in size on further standing to several times the size of the granules. These cells exhibited active amoeboid movement. (Plate II, Fig. 16). The mitochondria were usually present in a compact group. Wright's stain colored the eosinophilic granules a clear pink or red, but the shape of the individual granules was usually not discernible. The nucleus, which was rather deeply basophilic and separated from the gran-
ules by a narrow band of clear blue cytoplasm, was often bi- or tri-lobed. (Plate II, Fig. 15).

Basophiles

The basophiles of reptiles are also larger than their counterparts in the blood of man. In supravital stains the spherical granules varied in size and intensity of staining, and were so numerous that they often obscured the nucleus. With Wright's stain the basophiles presented much the same appearance as with the supravital stains. The nucleus was poorly defined, and much less basophilic than the granules. The latter were round, of various sizes in the same cell, and although most of them were deeply basophilic, some had a reddish tinge, denoting polychromatophilia. (Plate II, Fig. 17).
PLATE I

Explanations of Figures

Photomicrographs of Wright's stained smears
Magnification, 1566 diameters

Fig. 2 Erythrocyte (with intracellular hemogregarine) and a thrombocyte. From a horned toad.

Fig. 3 Two different sized monocytes from a Gila monster.

Fig. 4 Four monocytes exhibiting different degrees of phagocytic power. The central one, which is disintegrating, has engulfed an erythrocyte. From a horned toad.

Fig. 5 Monocyte (which has engulfed an eosinophile) from a Gila monster.
PLATE II

Explanations of Figures
Camera lucida drawings

Fig. 6  Myeloblast from horned toad. Wright's stained smear. x1500.

Fig. 7  Myelocyte from horned toad. Wright's stained smear. x1500.

Fig. 8  Myelocyte from swift. Wright's stained smear. x2500.

Fig. 9  Thrombocyte from Gila monster. Wright's stained smear. x2500.

Fig. 10 Medium-sized lymphocyte from Gila monster. Wright's stained smear. x2500.

Fig. 11 Medium-sized lymphocyte from horned toad. Wright's stained smear. x1500.

Fig. 12 Monocyte from Gila monster. Wright's stained smear. x2500.

Fig. 13 Monocyte with red granules from Gila monster. Wright's stained smear. x2500.

Fig. 14 Monocyte with red granules from horned toad. Wright's stained smear. x2500.

Fig. 15 Eosinophile from Gila monster. Wright's stained smear. x2500.

Fig. 16 Eosinophile from horned toad. Neutral red supravital preparation. x1500.

Fig. 17 Basophile from Gila monster. Wright's stained smear. x2500.
PLATE II
Parasitism

Intestinal parasites were found in some animals, blood parasites in others, and both types in still others. Hemogregarines (undetermined species), in the cytoplasm of the erythrocytes, occurred in one horned toad, (Plate I, Fig. 2), one Gila monster, and two of the bull snakes. The blood of three of the snakes and two of the Gila monsters contained blood-inhabiting nematodes (undetermined species of microfilariae). Adult filariae were found in the atria, pericardial cavity, and dorsal aorta of one of the Gila monsters. Cestodes (of undetermined species) were recovered from the small intestine of the ring-necked snake and one horned toad. Nematodes were found in the colon of the gecko, the small intestine of the ribbon snake and one desert scaly swift, and stomach of several horned toads. Only two species of these nematodes have been fully classified, Ophidascaris filaria from the ribbon snake, and Physaloptera phrynosoma from the horned toads and the swift.*

These records are given in Table VIII. The presence of an eosinophilia in certain of the parasitized animals has also been indicated.

*Species determined by Mr. C. A. Hannum.
## TABLE VIII

Records of Parasitism

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<td>S-7</td>
<td>Microfilariae</td>
<td>Blood</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S-5</td>
<td>Nematode</td>
<td>Small intestine</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>S-6</td>
<td>Nematode</td>
<td>Small intestine</td>
<td>(one worm)</td>
<td>(Basophilia?)</td>
</tr>
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<td></td>
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<tr>
<td>L-11</td>
<td>Nematode</td>
<td>Stomach</td>
<td>(one worm)</td>
<td>(Basophilia?)</td>
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-38-
DISCUSSION

No work has been reported heretofore on the blood of the geckos. The data on the blood of these small animals are not entirely satisfactory, but give an indication of relative position among the groups studied in regard to erythrocyte and leucocyte numbers, the sizes of erythrocytes and leucocytes, and percentages of types of leucocytes.

The average number of erythrocytes and the hemoglobin content for the Gila monsters were slightly lower than those given by Edwards and Dill (1935). They calculated the hemoglobin from the oxygen combining power of the blood and gave it as about one-half that of man, or presumably about eight grams per 100 cc. In their study two lots of animals were used, the first lot obtained through a dealer in San Diego, California, in the early fall of 1932 and the second lot from a dealer in Phoenix, Arizona, the following spring. The animals were bled shortly after they were received, so none was used during the winter. The lowest erythrocyte numbers and amounts of hemoglobin of this study were from animals bled during the winter period. This seems to indicate a lower oxygen combining power of the blood during the winter, which is logical because the animals were in a more or less inactive state.

No previous work has been reported on the numbers of
erythrocytes and the hemoglobin content per unit volume of blood in the horned toad. The cells of the circulating blood were mentioned only incidentally by Jordan and Speidel (1929) in their work with the horned toad.

The erythrocyte numbers of two species of snakes, *Coluber constrictor* and *Heterodon contortrix*, as reported by Baker and Kline (1931) may be said to correspond with the results of this study within the range of individual and species differences, although neither were the techniques of these two studies comparable nor the same species used.

The average numbers of erythrocytes for the swifts used in this study are somewhat lower than reported by Weigmann (1932) for the Old World lizard, *Lacerta vivipara* Jacq, although the numbers of two of our specimens exceeded Weigmann's average figures. He found greater numbers of erythrocytes per unit volume of blood during the winter period and concluded this was due to a decrease in the water content of the plasma. Thus the increase in erythrocyte numbers was merely a relative one; the total numbers of erythrocytes probably remained constant. These results do not correspond with those from the Gila monster which may indicate, in these two species of lizards, fundamental physiological differences.

From a consideration of Figure 1 it will be noted that the concentration of hemoglobin in the erythrocytes
of the Gila monsters is apparently greater than that in the erythrocytes of the swifts. This apparent difference in concentration is somewhat compensated for by the smaller size of the cells of the swifts. In other words, as the relations between numbers of erythrocytes and amounts of hemoglobin are reversed in the two groups (with the horned toads and snakes occupying intermediate positions), there is a corresponding decrease in size of the erythrocytes as shown in Table III.

E. B. Krumbhaar has stated: "The size of erythrocytes varies considerably in mammals, as well as in other orders, for reasons that are not clear. As the surface area of all the erythrocytes of the body is assumed to be proportional to the metabolic needs of the animal, in mammals the size should be in inverse ratio to their number; while this holds true between hot- and cold-blooded species, it does not seem to within the mammalia."* Maximow and Bloom (1930) also stated that in the lower vertebrates the numbers of erythrocytes vary according to species, usually inversely as to size. Comparing the small size of the erythrocytes of man (7.14 to 8.3 micra as given in Cowdry-1928), and their relatively large numbers per cubic millimeter (5,000,000) with those of the reptiles studied (Table III) we see that the results of this study support

*Special Cytology (Edited by E. V. Cowdry) 1928. Page 283.
Krumbhaar's statement regarding the relation between poikilothermic and homiothermic species. Although, as above stated, an inverse ratio between size and numbers of erythrocytes does not hold true in the Mammalia, Table III indicates that it may hold true among the groups of reptiles reported in this paper. Further work is necessary to establish this relationship, especially since the geckos do not seem to conform to this generalization, perhaps due to the incomplete data.

It is interesting to note that field observations as to the activities of the animals studied coincide with those on metabolic activity as indicated by the hemoglobin content of the blood. The geckos and Gila monsters would certainly be considered as the least active of the groups since the geckos are lethargic animals, at least during the daytime, and are seen abroad only at night. The Gila monsters are notoriously sluggish in their movements from place to place, although they are capable of rather quick movements of the head when snapping. The lightning-like movements of the swifts and their ability to dart away almost as rapidly as the eye can follow probably denote the highest metabolic activity of the groups in question. The horned toads and snakes would occupy relatively intermediate positions as indicated. Although the horned toads are capable of rather rapid movements, the snakes are capable of more sustained activity.
From a cytological point of view the reptilian erythrocyte differs from the mammalian erythrocyte mainly in the possession of a nucleus. It differs also in shape, the reptilian erythrocyte being ellipsoidal in contrast to the round, disc shaped, erythrocyte of most mammals, and in the possession of mitochondria. In the smears there were often erythroplastids of both round and oval shapes, apparently not formed by extrusion of the nucleus but by constriction of the cytoplasm and consequent division.

It was also quite evident that young erythrocytes matured in the blood stream because of the direct transitional series from the round, polychromatophilic cells with large, vesicular nuclei, to the definitely mature cells. The change in shape from round to oval has been noted by Maximow and Bloom (1930) in the development of the mature erythrocytes of reptiles. Amitotic reproduction was evident to a slight extent, with all intermediate stages present. Jordan and Speidel (1929) reported bi-nucleated erythrocytes, indicating amitosis, from the spleen of the horned toad. From the descriptions of the erythrocyte series recorded by Alder and Huber (1923) from several species of amphibians and reptiles, Jordan and Baker (1927) from the frog, Dawson (1931 d) from Hecturus, and Jordan (1932) from Proteus anguineus, as well as from the above statements, it would seem that differentiation and maturation of erythrocytes in the circulat-
ing blood occurs in many of the members of the Amphibia and Reptilia.

The difficulty in distinguishing free nuclei of disintegrated erythrocytes from small lymphocytes in smears of avian blood was pointed out by Blain (1928), who used supravital technique in preference to stained smears. Little difficulty has been encountered with such distinctions in reptilian blood smears if the smears were promptly and properly made. However, if the smears were made only a minute or so after the blood was drawn, variable numbers of pinkish blotches representing disintegrated erythrocytes appeared in the permanent mounts. Even the well preserved smears contained a few such blotches indicating that disintegration of the erythrocytes takes place in the blood stream. Jordan (1925) reported that erythrocytes of the frog may suffer dissolution in the peripheral blood. The few small vacuoles present in reptilian erythrocytes, as shown by neutral red staining, were similar to those described in the erythrocytes of the blood of the adult domestic fowl by Cook and Dearstyne (1934), and in the chick by Kelly and Dearstyne (1935). It seems probable that they represent a type of "segregation apparatus" as described by Evans and Scott (1921). Jordan (1925) found them, with supravital stains, in all the blood cells of the frog and considered them, as did Shipley (1919), to be related to the fundamental
metabolic activities of the cells.

Nittis (1930) described a 'stigma' or bright blue spot in the nucleated erythrocytes of certain of the lower vertebrates when the cells were supravitally stained with brilliant cresyl blue. Dawson (1931 a) (1931 b) has shown these spots to be present in the erythrocytes of several other of the lower vertebrates including the horned toad. He has also described (1931 c) vacuoles in atypical erythrocytes from the blood of Necturus which were not stainable either supravitally or in Wright's stain. He suggested that they may have been degenerative in nature, although he stated that such vacuoles usually show marked affinity for vital dyes. Such 'stigmata' appeared in the erythrocytes of the reptiles used in this study when stained with neutral red, which suggested that perhaps they were merely the first vacuoles of the so-called "segregation apparatus" to appear. The non-stainable vacuoles described by Dawson, were also seen in the erythrocytes of the blood of certain of the reptiles.

The thrombocytes, as stated by Maximow and Bloom (1930) apparently take the place of the blood platelets of mammals. It has been suggested by Jordan and Speidel (1924) that the minute, metachromatic granules in the cytoplasm of the thrombocytes of the urodeles are similar to those of the hemogenic megakaryocytes of mammals. It was probably with this in mind that they later (1929)
identified the thrombocytes with thrombus formation in the horned toad. Maximow and Bloom (1930) identified the agglutination and disintegration of the thrombocytes with the formation of centers of thrombin elaboration.

Sugiyama (1926) described the thrombocytes as differentiating from the same ancestral cell type as the erythrocytes in the chick. Cook and Dearstyne (1934) accepted this view and stated that thrombocytes were very numerous in avian blood. Although they did not include the thrombocytes in the differential leucocyte counts, they may have included them in the total counts. Jordan (1935), however, in his work on the blood of birds stated that the thrombocytes developed from small lymphocytes, which was in agreement with his former work on urodèles (Jordan and Speidel 1924, and Jordan, 1932). Although Loewenthal (1928, 1929, 1930, 1931) has done a great deal of work on the leucocytes of amphibians and reptiles, he has not included thrombocytes in the differential leucocyte counts. Sabrazes and Muratet (1924 a) did not include the thrombocytes in the differential leucocyte counts of either the chameleon, Chameleon vulgaris, or the wall lizard, Lacerta muralis (1924 b). In the present study, the thrombocytes were considered as leucocytes because of cytological similarities, and were included in the differential counts. Although the group averages (Table VI) showed that over one-half of the leucocytes were
thrombocytes, in some animals the numbers were very small, and in one horned toad no typical thrombocytes were seen.

The characteristics of the lymphocytes of reptiles and other lower vertebrates are fairly well established. The lymphocytes of avian blood were described by Lewis and Lewis (1926), Sugiyama (1926), Blain (1928), and Cook and Dearstyne (1934). These cells of reptilian and amphibian blood have also been amply discussed by Jordan and by Loewenthal in their various publications cited. In addition, both of these workers have described the lymphocytes in the blood of representative fish. Since this study was not concerned with the hemogenic functions of the lymphocytes, there is little to add to the literature concerning the lymphocytes. Although Jordan (1925) reported only occasional relatively large globules appearing in the lymphocytes of the from in supravitai stains, one or two such large globules were present in almost every lymphocyte examined in this study after the preparations were several hours old. Alder and Huber (1923) stated that the lymphocytes of reptiles were not comparable to those of mammals. From morphological appearance in the stained smears, as well as in supravital stains, the lymphocytes studied here were apparently identical with those of mammals although they were slightly larger.

The striking characteristics of the monocytes as shown in the supravital preparations were the formation
of very large, irregular, deeply stained vacuoles in the cytoplasm. It seemed probable that such reactions were phagocytic in nature, and consisted of active ingestion and storage of the dye rather than a passive staining of preformed globules, as indicated by Jordan (1925) in the blood cells of the frog. The phagocytosis of abnormal erythrocytes and of eosinophils by monocytes in vivo, as indicated in Plate I, Figs. 4 and 5, supported the above observations made in vitro. That the monocytes may become extremely active under certain conditions was indicated also by the work of Jordan and Speidel (1928) on splenectomized horned toads.

Conflicting reports are to be found concerning the presence of polymorphonuclear neutrophiles in the blood of reptiles. Such cells were found in amphibians by Jordan and Speidel (1924), Jordan (1925), and Loewenthal (1928, 1929, 1930), but in birds the reports of Doan, Cunningham, and Sabin (1925), Sugiyama (1926), Blain (1928), Maximow and Bloom (1930), Cook and Dearstyne (1934), and Kelly and Dearstyne (1935), indicate that the polymorphonuclear leucocytes were eosinophilic rather than neutrophilic. For reptiles, von Werzberg (1911) concluded that typical neutrophiles were not present, but Loewenthal (1931), after having used several methods of staining, disagreed. Jordan and Speidel (1924) stated that no neutrophiles occurred in birds or reptiles, nor finely,
granular leucocytes of any sort, although they described (1929) a few cells of the horned toad that corresponded with the neutrophilic granulocytes of Lacerta muralis and Tarentola mauritanica, as described by Alder and Huber (1923). Sabrazes and Muratet (1924 a) described polymorphonuclear leucocytes of the chameleon with horseshoe shaped nuclei, but "a granulations peu apparentes". They stated in a report on the leucocytes of the blood of Lacerta muralis (1924 b) that they did not find granular leucocytes of a polymorphonuclear neutrophilic type, but did describe monocytes with azurophilic granules. Such azurophilic granules have been described for mammalian lymphocytes by Cowdry (1928), and for amphibian lymphocytes by Jordan (1932). In Wright's stain there were present, as noted in the cell descriptions, very fine red granules in certain monocytic cells, which granules were probably the same as the 'azurophilic' granules of the above workers. If such were the case, it would seem probable that the monocytes with the fine red granules were of lymphoid origin, and could not be considered as typical granular leucocytes. In certain smears complete transitional series could be found from lymphocytes to monocytes, and to the monocytoid cells with red granules and polymorphic nuclei. (Plate II, Figs. 12-14).

The eosinophiles of certain reptiles were well described by Loewenthal (1909)(1931). Although Alder and
Huber (1923) described two types of eosinophiles in reptiles, one with round granules and the other with cylindrical granules, and Jordan (1924) described a coarsely and a finely granular eosinophilic type in the skate. Jordan and Speidel (1929) described but one type in the horned toad. Sabrazes and Muratet (1924 a) described the granules of the eosinophiles of Chameleon vulgaris as both round and rod-like in form. As was pointed out in the cell descriptions, the fusiform granules may round up in the supravital preparations of living cells, so that both types of granules may occur in the same cell. Thus, it is possible that cells with round granules, and cells with fusiform granules, might be considered as different types of cells, even though they belonged to the same cell type.

The possibility that the production of eosinophiles might be influenced by heavy infestations of parasitic worms inhabiting the digestive tract is indicated by Table VII. Although an eosinophilia usually was found coincident with the intestinal infestations, such was not the case with the blood-inhabiting parasites. In this connection an observation of Jordan and Speidel (1924) is of interest. They stated that during the metamorphosis of the larval frog the eosinophiles were more numerous than the neutrophiles in the region in which the gut was being resorbed, while in the region where the tail tissues were
being resorbed, the reverse was true.

The significance of basophiles in vertebrate blood is uncertain, although their morphology has been described by many workers. For the reptiles, the morphological descriptions by Jordan and Speidel (1929), Loewenthal (1930) (1931), Alder and Huber (1923), and Sabrazes and Muratet (1924 a) (1924 b) are similar. The consensus of opinion probably would be that these cells were products of abnormal changes in developing eosinophiles since the granules were often metachromatic and the nucleus usually like that of a degenerating cell. Cook and Dearstyne (1934) and Kelly and Dearstyne (1935) have reported that mitochondria were rarely seen in the basophiles of fowls, but reported the presence of vitally stainable granules and globules. In this study no mitochondria have been seen in reptilian basophiles and only rarely were there any vacuoles stained in neutral red. The nuclei of the basophiles have been compact and spherical, or slightly indented, but not polymorphic. They were defined poorly in Wright's stain, so it would seem that these nuclei were probably degenerate. It was notable from the differential leucocyte counts that large numbers of basophiles were present in the geckos. Since several of these animals harbored intestinal parasites and showed coincident eosinophilia, it is possible that such 'basophilia' may indicate cell relations between the eosinophiles and
basophiles.

Immature cells, denoting various stages in the differentiation and development of the above definitive cell types, were found in all of the slides. The presence of such cells was probably more noticeable in the blood of the swifts than in any other group studied. Since this problem did not include the study of the origin and development of the blood cells, only two outstanding types of immature leucocytes are shown; namely, a myeloblast from the blood of a horned toad, and myelocytes from the blood of a horned toad and from the blood of a swift. (Plate II, Figs. 6-8).

Although wide variations were found in the individual blood pictures, there was certainly no evidence to support the statement of Sabrazes and Muratet (1924 b) that since the blood pictures of reptiles varied so greatly, one would have to conclude that each genus, or even species, possessed a separate and distinct hematological type, which varied, moreover, under a host of physiological and pathological conditions.
SUMMARY AND CONCLUSIONS

1. Determinations were made of number of erythrocytes and leucocytes, and amount of hemoglobin per unit volume of blood for five groups of reptiles: geckos, Gila monsters, horned toads, snakes, and swifts.

2. There was a positive correlation between the amount of hemoglobin and number of erythrocytes per unit volume of blood in the animals studied.

3. The unit quantity of hemoglobin present showed a positive correlation with the normal activities in each of the different groups of animals.

4. The types of blood cells were similar to those of man with the following exceptions: the erythrocytes were nucleated; thrombocytes were found in place of blood platelets; no true neutrophiles were found.

5. For the corresponding leucocytes, the cell sizes were somewhat larger in reptiles studied than in man.

6. Heavy infestations of stomach or intestinal nematodes apparently increased the percentage of eosinophiles in the blood stream of the parasitized animals.
7. These data fail to support the conclusion of Sabrazes and Muratet (1924 b) that each genus, and possibly each species of reptiles, has a distinct hematological picture. In spite of wide variations among individual hematological pictures, there was a close resemblance, in generalized pictures in all groups studied.
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