

Malarial Parasites of the "Jesu Cristo" Lizard
Basiliscus basiliscus (Iguanidae) in Panama

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Malarial Parasites of the "Jesu Cristo" Lizard *Basiliscus basiliscus* (Iguanidae) in Panama

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SYNOPSIS. The iguanid lizard *Basiliscus basiliscus* in Panama is parasitized by *Plasmodium basilisci* and *P. achiotense* sp. nov. *P. basilisci* in this host is characterized by schizonts containing 4-14 merozoites, with schizonts parasitizing proerythrocytes containing more merozoites than those in erythrocytes. Asexual parasites lack cytoplasmic projections, while mature gametocytes are round or oval with regular margins.

THE malarial parasites of Panamanian basilisk lizards have not been described previously, altho reference was made in the Annual Report of Gorgas Memorial Laboratory for 1964 (1) to the presence of *Plasmodium basilisci* Pelaez and Perez-Reyes in *Basiliscus vittatus* and *B. plumifrons* collected in the vicinity of Almirante, Bocas del Toro Province, Panama. During my survey of hemoparasites of Panamanian lizards, I encountered 2 species of *Plasmodium* in *Basiliscus basiliscus*, one of which can be assigned to *P. basilisci*, and one of which is undescribed. In view of certain differences of the Panamanian *P. basilisci* between strains reported from Mexico (4), British Honduras (2) and El Salvador (2), it is described in detail below.

MATERIALS AND METHODS

Basilisks were collected live by hand or from mammal traps in various localities of central and eastern Panama, and brought to Gorgas Memorial Laboratory in Panama City for examination. Thin smears, prepared by clipping toes of lizards, were fixed in absolute methanol and stained for 1-1.5 hr with a 1:10 dilution of Giemsa at pH 6.8. Slides were examined at 600 \times and 1,000 \times oil immersion, and photomicrographs were taken at 1,000 \times . Strain isolations were made by inoculation of blood from infected lizards into juvenile basilisks which had remained negative for blood parasites for 3-6 months in the laboratory. Descriptions below are based primarily upon study of natural infections followed for 1-6 months in the laboratory. Measurements of parasites are in micra. Schizonts were considered to be "apparently mature" when segmentation seemed to be imminent or was observed. Mean differences were evaluated by the Student t-test, with $p \geq 0.05$ considered significant, and $p \geq 0.01$ highly significant.

RESULTS

Plasmodium basilisci from *Basiliscus basiliscus* in Panama

The description which follows is based upon 4 natural infections, the hosts of which were collected from these localities: El Aguacate, Panama Province; Madden Forest, Canal Zone; Achiote, Colon Province; and Sasardi, San Blas Territory.

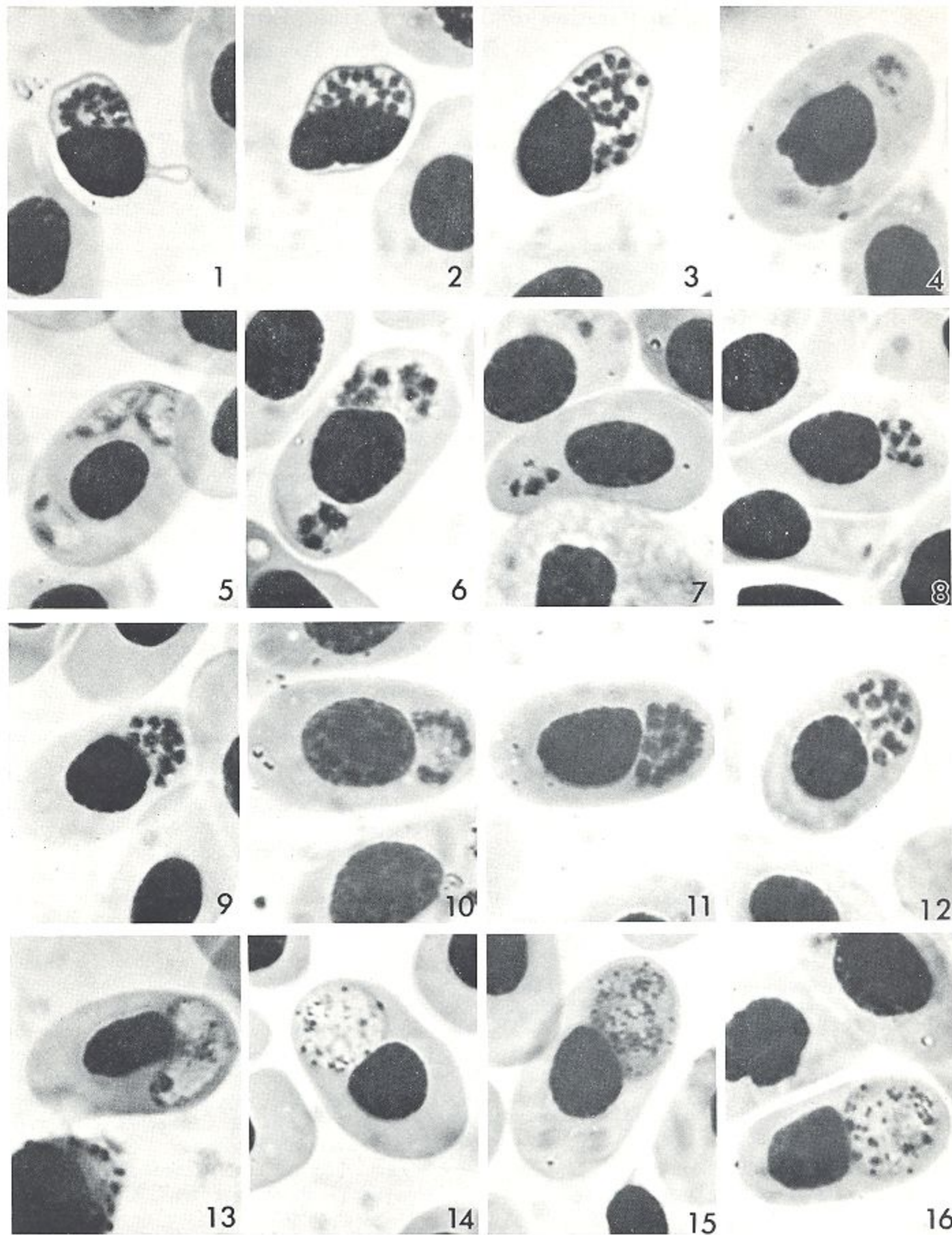
P. achiotense is characterized by the combination of prominently pigmented, large schizonts containing 36-56 merozoites and oval or round gametocytes which are about $\frac{1}{2}$ larger than those of *P. basilisci*.

EE-schizonts of *P. basilisci* were observed commonly in thrombocytes and occasionally in lymphocytes, and appeared early in experimental infections induced by blood inoculation.

Trophozoites. The smallest stages are approximately 1 in diameter, squarish, with no visible cytoplasm; medium-blue cytoplasm becomes visible when the trophozoite reaches 2 by 2 (Fig. 4); no vacuole or pigment is present. Larger trophozoites are rather elongate, with pigment represented by a minute, dark dot.

Schizonts. The smallest binucleate schizonts are 4 by 3, with scanty pigment, deep red nuclei, medium blue cytoplasm and no vacuole (Fig. 5). Apparently mature schizonts (Figs. 7-12) range in size from 4-8 by 3-6, \bar{x} 5.2 \pm 0.1 by 3.8 \pm 0.1 (N = 100). Merozoites range from 4-14; means from individual infections are 9.4 \pm 0.4 (N = 25), 8.5 \pm 0.4 (N = 25); 6.6 \pm 0.5 (N = 25), and 4.3 \pm 0.2 (N = 25). The variation in means is correlated with the type of host cell, rather than representing geographic variation: the mean number of merozoites from schizonts in proerythrocytes (Figs. 10-12) is 9.4 \pm 0.3 (N = 45), while that from schizonts in erythrocytes (Figs. 7-9) is 5.5 \pm 0.3 (N = 55), a highly significant difference ($t = 9.960$). Schizonts are usually (80%) fan-shaped; occasional schizonts are elongated, or have nuclei arranged in rosettes or morulae. Pigment, deep golden in hue, is always present, but in lesser amount when proerythrocytes are parasitized.

Gametocytes. Immature gametocytes are oval or slightly elongate (Fig. 13), often with irregular borders. Dark golden pigment granules are loosely aggregated to form a central mass. As gametocytes mature, they become oval (usually, Fig. 15) or round (Figs. 14, 16), with smooth borders. Pigment granules, 10-20 or so are scattered over the cytoplasm, and are always present. Mature gametocytes range from 6-10 by 5-8, \bar{x} 8.0 \pm 0.1 \times 6.6 \pm 0.1 (N = 50). Macrogametocytes (Figs. 15, 16) stain deep blue; their nuclear area is a prominent pink, as is the nucleolus, which is usually rather small. Microgametocytes (Fig. 14) stain deep pink, and the nuclear elements appear concentrated in one area of the parasite.



Exoerythrocytic Stages. In all infections studied, unsegmented schizonts containing 10-16 nuclei were commonly seen in thrombocytes (Figs. 1-3). A series of 11 averaged 12.8 ± 1.2 nuclei. In experimental infections established by blood inoculation, EE-schizonts appeared early following patency and persisted thruout the course of infections. Occasional schizonts were seen in lymphocytes, but most were in thrombocytes. No parasites have been seen in fixed cells of sectioned tissues.

Types of Host Cells Parasitized. In 4 infections studied, schizonts were found in proerythrocytes (76, 76, 20, and 49%) and erythrocytes only. Young parasites reflected similar distributions in the respective infections. A single trophozoite was seen in a stem cell. In 2 infections, gametocytes occurred in erythrocytes (34 and 100%) or proerythrocytes.

Effects upon Host Cells. Young asexual parasites had no discernible effect upon host cells. In 3 infections, schizonts caused no distortion of either host cell or its nucleus; in 2 infections 4% of the nuclei were displaced slightly. In one infection, where both schizonts and gametocytes occupied proerythrocytes primarily, schizonts distorted both host cell (80%) and its nucleus (96%), and often (48%) displaced the latter. In this infection, gametocytes also distorted the cell (92%) and its nucleus (100%), and displaced the nucleus (84%). Distortion usually resulted in apparent disorganization of the nucleus. In an infection which was confined to erythrocytes, the effects were different; distortions of cell (32%) and nucleus (40%) were less common, and the nucleus less often displaced (36%). In this infection the distortion was due to the parasite pushing against the nucleus, with no obvious disorganization of its contents. Segmenters were usually polar (93%) in position, and less often lateropolar (5%) or lateral (2%), among all infections. Gametocytes were polar (98%) or lateropolar in position.

A 2nd, very different *Plasmodium* was found occasionally in *B. basiliscus* which differs from all known species. Accordingly, I designate it

Plasmodium achiotense sp. nov.

Diagnosis. A species of *Plasmodium* which produces large oval, round or elongate schizonts containing 36-56 nuclei, and oval or round gametocytes. Both schizonts and gametocytes contain prominent dark golden pigment granules, aggregated into irregular central masses in schizonts and dispersed as 30 or more granules in gametocytes. Para-

sitized erythrocytes are hypertrophied and distorted, and their nuclei are displaced and often distorted.

Description: Trophozoites. The smallest trophozoites are 2 by 1.5, roughly triangular in shape, with the squarish nucleus bordered on 2 sides with a small amount of very pale blue cytoplasm; pigment is lacking but an indistinct vacuole is usually present. As the trophozoite grows to 2 by 3, it elongates and the deep red nucleus appears band-shaped (Fig. 17). A minute, dark dot of pigment is present in trophozoites which are 4 by 3.

Schizonts. Binucleate schizonts 5 by 3 were observed; as nuclei divide, the schizonts become rounded (Figs. 18-20) and most nuclei assume a peripheral distribution. Pigment granules aggregate into 1-3 dark golden, adjacent clumps which are usually centrally located. These often fuse into a large, irregular mass.

Apparently mature schizonts (Figs. 21-24) are 10-18 by 9-13, \bar{X} 13.2 ± 0.4 by 10.9 ± 0.2 in size, and contain 36-56, \bar{X} 46.8 ± 1.1 ($N = 25$) squarish nuclei. Their shape is usually oval but may be round or elongate. Pigment is always present, and prominent.

Gametocytes. Immature gametocytes are usually elongated, with pigment granules loosely aggregated into a single, peripheral, golden mass. Mature gametocytes (Figs. 25-28) are oval or round, with dark gold pigment granules scattered generally throughout the cytoplasm. Dimensions range from 9-13 by 8-11, \bar{X} 10.9 ± 0.1 by 9.6 ± 0.1 ($N = 50$). Macrogametocytes (Figs. 25, 26) stain pale to medium blue; their nuclei are represented by pinkish areas larger than the pigment mass; a single nucleolus is usually evident. Microgametocytes (Figs. 27, 28) are unstained; an occasional pink hue possibly results from diffuse nuclear elements; no nucleoli were observed.

Exoerythrocytic Stages. No exoerythrocytic stages were seen altho an infection was followed for 3 months in the laboratory. Tissues were not examined for this species.

Types of Host Cells Parasitized. All parasites observed were in erythrocytes.

Effects upon Host Cells. Both schizonts and gametocytes distort host cells and displace their nuclei (98-100%). Host cell nuclei are often distorted (32-44%). Host cells are hypertrophied, usually in their width. Position of schizonts is usually lateral (88%) to the host cell nucleus, while gametocytes are often polar (68%).

Figs. 1-16. *Plasmodium basilisci* from *Basiliscus basiliscus*.

Figs. 1-3. Schizonts in thrombocytes.

Fig. 4. Trophozoite in erythrocyte.

Figs. 5-6. Immature schizonts in erythrocytes.

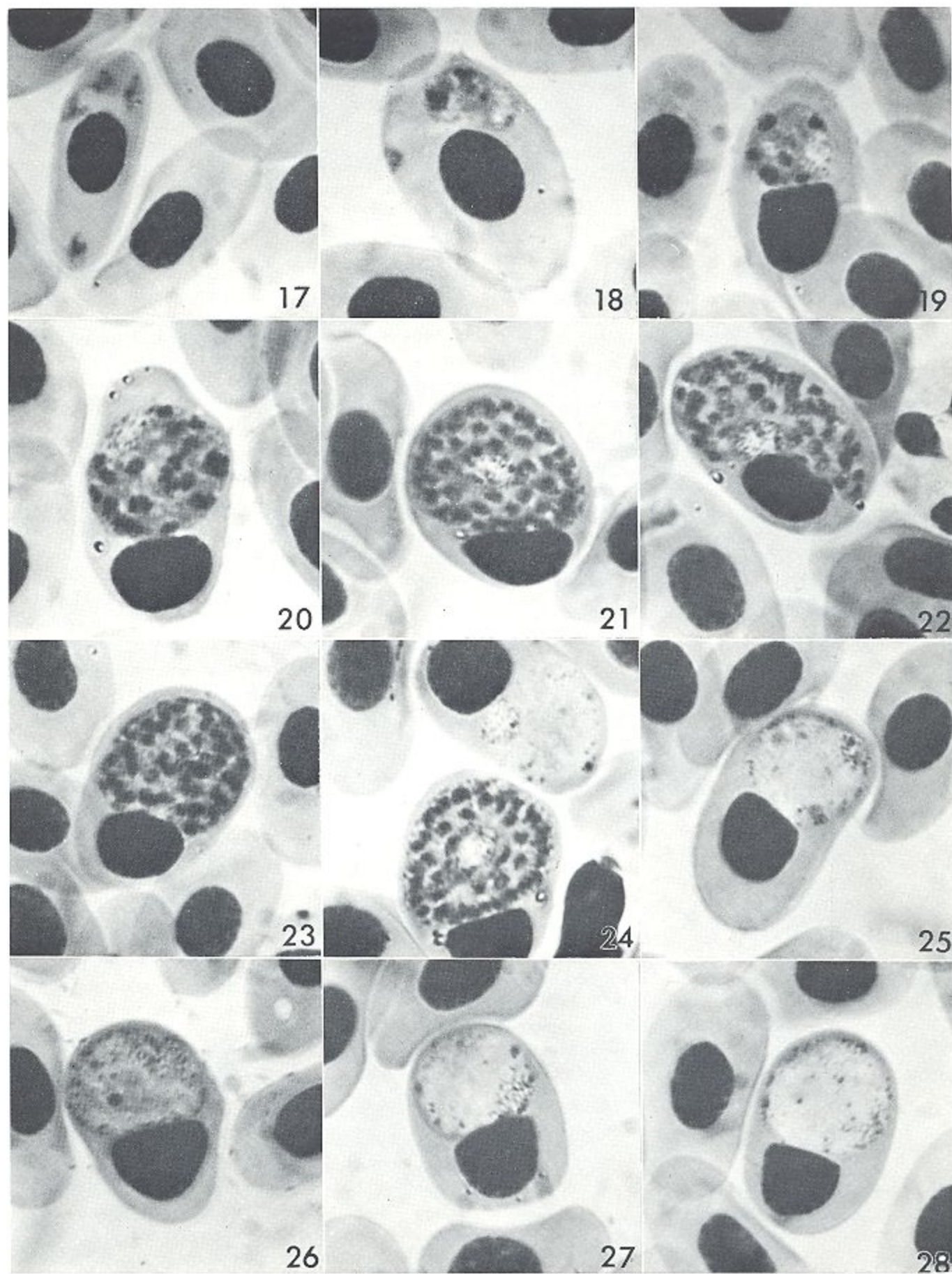
Figs. 7-9. Mature schizonts in erythrocytes.

Figs. 10-12. Mature schizonts in proerythrocytes.

Fig. 13. Nearly mature ♂ gametocyte in erythrocyte.

Fig. 14. Mature ♂ gametocyte in erythrocyte.

Fig. 15, 16. Mature ♀ gametocytes in erythrocyte and proerythrocyte respectively.



Type Host: *Basiliscus basiliscus* (Linnaeus) (Sauria, Iguanidae).

Type Locality: Achiote, Colon Province, Republic of Panama.

Geographic Range: Known only from the type locality.

Location of Types: The type slide is retained at present in my collection. Paratypes are deposited in the Dept. of Zoology, Univ. of California, Los Angeles, and with Prof. P. C. C. Garnham, Imperial College Field Station, Silwood Park, England.

Incidence and Levels of Parasitemia. Altho incidence studies on the Panamanian saurian malarial parasites are not yet completed, preliminary examinations revealed an overall infection rate for *P. basilisci* of 23%. In the Achiote area its prevalence is higher; approximately 50% of the basilisks were infected with *P. basilisci*. *P. achiotense*, which was found only at Achiote, parasitized about 20% of the lizards collected from June to August but was not detected in other months. About half of the lizards infected with *P. achiotense* also were parasitized by *P. basilisci*.

Parasitemias in *P. basilisci* natural infections usually were at levels not exceeding 100 parasites/10,000 erythrocytes; the highest parasitemias recorded were 1,065 and 1,025/10,000 erythrocytes. Experimental infections of *P. basilisci* did not exceed 200 parasites/10,000 erythrocytes. The maximum parasitemia of *P. achiotense* observed was 232/10,000 erythrocytes, in the single acute infection obtained.

DISCUSSION

Plasmodium achiotense is similar to 2 other New World saurian malaria parasites, *P. cnemidophori* Carini and *P. balli* Telford, in that it has very large schizonts. It is readily distinguished from both by its consistently oval to round gametocytes; those of *P. cnemidophori* are elongate, while *P. balli* usually has elongated, thick gametocytes. *P. balli* usually lacks pigment; it is never prominent. That of *P. cnemidophori* is dark rather than golden as in *P. achiotense*. The appearance and dimensions of *P. achiotense* are very different from those of the other New World sauramoebas: *P. diploglossi*, *P. floridense*, *P. mexicanum*, *P. tropiduri*, *P. beltrani*, *P. brumpti*, *P. gonatodi*, *P. morulum*, and *P. aurulentum*. Gametocytes of *P. achiotense* are more likely to be confused with those of *P. basilisci* than with those of any of the sauramoebas, but those of *P. basilisci* are about $\frac{1}{2}$ smaller in both length and width, and have about half as many pigment granules dispersed in the cytoplasm.

Plasmodium basilisci in *Basiliscus basiliscus* differs from

P. basilisci as described from *Basiliscus vittatus* by Pelaez and Percz-Reyes (4) from Veracruz, Mexico and by Garnham (2) from British Honduras in 3 important characters. Merozoite number in *B. vittatus* varies from 4-8; in *B. basiliscus* it ranges from 4-14. Higher counts in the latter species reflect parasitization of proerythrocytes. More merozoites in schizonts parasitizing immature erythrocytes have previously been recorded for *P. sasai* (7) and *P. aurulentum* (6), testifying to the necessity for considering erythrocyte maturity when stating the number of merozoites characteristic of saurian malarial parasites. Both Pelaez and Perez-Reyes (4) and Garnham (2) reported cytoplasmic processes in asexual parasites, and irregularity in outline of gametocytes, which are described as ovoid or elongated. *P. basilisci* as described here did not have cytoplasmic processes at any stage, and irregularity of gametocyte margins was observed only in immature gametocytes. Mature gametocytes are consistently round or oval, never elongated, in the Panamanian lizards.

Herban and Coatney (3) reported *P. basilisci* from *Iguana iguana* of El Salvador to have 6-9 merozoites in schizonts, with an average of 7. They also described macrogametocytes as elongate, and microgametocytes as irregularly shaped.

Production of circulating EE stages in experimental infections established by blood inoculation is known from only 2 other saurian malaria parasites, *P. mexicanum* (8) and *P. morulum* (5). Exoerythrocytic schizonts have not been reported previously for *P. basilisci*.

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Figs. 17-18. *Plasmodium achiotense* from *Basiliscus basiliscus*. All host cells are erythrocytes.

Fig. 17. Trophozoites.

Figs. 18-20. Immature schizonts.
Figs. 21-24. Mature schizonts.
Figs. 25-26. ♀ gametocytes.
Figs. 27-28. ♂ gametocytes.