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Effect of Temperature upon Reproduction and Cyclic Development of Culture Forms of Trypanosoma Avium

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EFFECT OF TEMPERATURE UPON REPRODUCTION AND CYCLIC DEVELOPMENT OF CULTURE FORMS OF *TRYPANOSOMA AVIUM*

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the Graduate Faculty

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Master of Science

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by

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APPROVED FOR THE GRADUATE FACULTY

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ACKNOWLEDGMENTS

The author wishes to express gratitude to Dr. Glen Clark for the selection of this problem and also for his guidance and assistance. Valuable suggestions were also made by Miss Janet Lowe and Dr. Philip Dumas. The author also extends appreciation to his wife, Myrna, for her assistance with the figures and typing of the manuscript.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Danilewsky, in 1885, was the first to describe avian blood parasites of the genus Trypanosoma. Laveran, in 1903, described a trypanosome which he found in an owl (4). He classified the organism as Trypanosoma avium; the species name has since been ascribed to a number of trypanosomes found in a variety of birds.

The genus Trypanosoma is composed of hemoflagellates of the suborder Trypanosomatina in the protozoan class Zoomastigophorea. They are characterized in part by having one flagellum, either free or attached to the body by means of an undulating membrane; a heteroxenous life cycle; and reproduction by binary or multiple fission, or both. Many are polymorphic, and all are parasitic.

The vectors of T. avium are probably mosquitoes and hippoboscids. Levine reports that the only complete life cycle for T. avium was worked out by Baker using organisms from rooks and jackdaws. The hippoboscid fly, Ornithomyia avicularia, serves as the vector in England. After being ingested with a blood meal, the trypanosomes assume the crithidial form in the midgut, undergo binary fission, and pass to the hindgut. After further multiplication, they enter a piriform stage which yields a small, metacyclic form. Birds are infected by eating the infected insects. Once in the bird the trypanosomes penetrate the upper digestive tract and invade the lymphatic system. They appear in the blood stream 18 to 24 hours after infection (11).

Though much research has been done on the parasitic protozoans of the suborder Trypanosomatina in general, particularly those patho-

genic trypanosomes which infect mammals, relatively little research has been directed toward those forms which infect birds. That the pathogenic species are of primary concern cannot be denied. This importance is emphatically shown by Hornby's (7) statement, "Trypanosomiasis is unique among diseases in that it is the only one which by itself has denied vast areas of land to all domestic animals other than poultry. The areas of complete denial are all in Africa and add up to perhaps one-quarter of the total land surface of this continent."

Although avian trypanosomes apparently cause little pathology (11), and are therefore not of prime concern medically or economically, they do remain an interesting group of parasites and present a wide area for investigation. With these thoughts in mind, the author decided to investigate the growth rates and developmental stages of Trypanosoma avium taken from the American robin, Turdus migratorius, and cultured in vitro.

LITERATURE REVIEW

General Morphology and Incidence

Novy and MacNeal (12) found hemoflagellates of the genus Trypanosoma in several birds of North America. They described both a short form and a larger form with myonemes. The larger forms ranged from 21μ to 40μ in body length, in the living state. Body width was between 5μ and 7μ. Stained specimens had a body length of 35μ to 65μ. The body length of the smaller forms ranged from 14μ to 25μ, in the living state. Width was between 3.5μ and 5μ. Stained specimens of the smaller forms varied in body length from 20μ to 25μ.

Coatney and Roudabush (4) made a survey of blood parasites in Nebraska birds. They, too, reported finding two distinct sizes in most species. They described both large, myonemed and small, non-myonemed forms. Coatney and Roudabush considered these organisms as belonging to T. avium.

Other species of avian trypanosomes described in the literature include T. calmettei, from the chicken in southeast Asia; T. gallinarum, from the chicken in central Africa; T. hannai, described from the pigeon; and T. numidae, from the guinea fowl (11). Blood parasites of the genus Trypanosoma have been found in over 21 other species of birds, but they are generally classified as T. avium.

Clark (3) studied the comparative morphology of four genera of the family Trypanosomatidae. In describing the general characteristics of the genus Trypanosoma (including T. avium), he listed the following features: presence of a short undulating membrane, a

kinetoplast (parabasal body) located slightly anterior to the nucleus and posterior to a small contractile vacuole, and a resevoir draining the vacuole which is lined by a membrane that is continuous with the flagellar sheath (determined with the aid of a photomicrograph).

Culture Techniques

Though trypanosomes occupy the circulatory and lymphatic systems in general, they seem to be most numerous in the bone marrow of birds. Diamond and Herman (6) developed a suitable technique for obtaining the marrow from living birds. Their data indicate the superiority of bone marrow over heart blood as a source of trypanosomes from the Canada goose.

The bone marrow is also preferable to both peripheral and visceral blood as a source of trypanosomes in the infected American robin, T. migratorius. Clark (2) prepared slides of peripheral and visceral blood and bone marrow taken from 70 adult robins. Forty-three birds showed trypanosome-infected marrows, whereas only one blood preparation was positive.

An excellent account of the media used to cultivate mammalian trypanosomes was presented by Tobie (13). As might be expected, the majority of work done on the development of suitable culture media has been directed toward these pathogenic species in mammals. Several media, however, have been developed which are applicable to avian trypanosomes.

Diamond (6) developed a diphasic medium, SNB-9 (saline-neopeptone-blood), for growing trypanosomes from frogs. In his study

on trypanosomes from geese, he and Herman tried this medium and a water-blood monophasic medium developed by Ponselle. They found the SNB-9 medium supported trypanosomes from geese, swans, and hawks. The monophasic medium was discontinued after negative results were obtained.

Krassner (8) studied the effect of temperature on growth of a lower Trypanosomatidae, Leishmania tarentolae, in a defined medium. In his work he investigated the effect of two antibiotics, penicillin and streptomycin, on the growth of this parasite of African lizards (antibiotics are often included in media to restrict bacterial contamination). Whereas 100 g of streptomycin in 2.9 ml of medium partly inhibited growth, he found that as much as 20,000 units of penicillin in 2.9 ml of medium showed no activity against the organism.

The media used in this particular study were prepared as described by Baker (1). In his study of avian trypanosomes, Baker used an NNN and 4N (i.e. "nutrient NNN") diphasic medium. Both of these media supported trypanosomes isolated from a rook, Corvus f. frugilegus.

Cyclic Development

Lehmann and Sorsoli (9) explored the cyclic development of culture forms of Trypanosoma ranarum (using Diamond's medium SNB-9), a hemoflagellate infecting amphibians. They reported slender crithidia, pear-shaped crithidia, leishman bodies, and disintegrating forms. All cultures were maintained at room temperatures (15-22°C). The original inoculum contained 64% slender and 19% pear-shaped crithidia. The slender crithidia began to decline and reached a low level on the fifth day (4-5%), whereas the pear-shaped crithidia had a high of 93% on the

fifth day. The cycle reversed after the fifth day. By 10-13 days the pear-shaped forms had reached a low of 3%, and the slender crithidia had peaked at 93%.

Lehmann (10) continued his investigation of cyclic development by observing the effect of various controlled temperatures on the development in vitro. Cultures maintained at 20°C and 25°C showed development similar to that previously described for cultures at room temperature. Pear-shaped forms reached a maximum of 64% on the second day and a minimum of 10% after the sixth day at 9°C. At 31°C the pear-shaped crithidia again reached a peak of 79% on the second day and fell to 26% of the total by the sixth day. Cultures grown at 35°C were negative after 24 hours.

DeBoe, McGhee, and Hanson (5) made a comparison of growth rates of T. cruzi and T. rangeli on a diphasic medium prepared with blood from ducks, chickens, rats, and rabbits. They found the maximum growth of these organisms on duck blood was comparable to the growth rate on mammalian blood. Trypanosoma rangeli grown on chicken blood reached a higher peak than those cultured on rat blood and reached a lower peak than those grown on rabbit blood. It appeared that the serum fraction in some ducks (the oldest ones) was trypanocidal.

MATERIALS AND METHODS

Trypanosomes for the original stock cultures were taken from the bone marrow of an adult robin collected in Kittitas County. Within an hour after the bird's death, the tibio-tarsus was removed and cleaned of adhering tissues. The bone was then flamed lightly before removing the proximal end with a pair of scissors, which had been sterilized in alcohol and flamed. A fine sterile probe was then inserted into the marrow cavity and rotated. Marrow adhering to the probe was transferred to the liquid phase of the culture medium.

The original organisms were isolated in an NNN culture at room temperature (24-28°C). The NNN medium was prepared following the traditional recipe described by Baker (1) in his study on avian trypanosomes. Media preparation was prepared in the following manner: NaCl, 6g; agar, 16 g; distilled water, 950 ml. Portions of this solution were placed in 5-ml, screw-cap tubes and autoclaved at 15 lbs of pressure for 15 min. After sterilization and subsequent cooling, 1 ml of sterile, fresh rabbit blood was added to each vial. This base was allowed to solidify at a slant, after which an overlay of 1 ml of sterile Locke's solution containing 2.86 mg of streptomycin sulfate were added to the slant. Locke's solution was prepared by combining: NaCl, 8 g; KCl, 0.2 g; $CaCl_2$, 0.2 g; KH_2PO_4, 0.3 g; glucose, 2.5 g; distilled water, 1 l. This solution was sterilized in an autoclave. The tubes containing the medium were then incubated for 24 hours at 37°C. After incubation they were checked for bacterial contamination and refrigerated. Media in which sheep or chicken sera were substituted for

rabbit serum were prepared in the same manner.

Organisms were cultured also in 4N ("nutrient NNN") medium prepared with the following ingredients in 1 liter of distilled water: proteose peptone, 15.0 g; yeast extract, 5.0 g; NaCl, 5.0 g; agar, 12.0 g. The solution had a pH of 7.4. Again, portions of this solution were dispensed in 5-ml tubes and autoclaved. Rabbit serum, Locke's solution, and streptomycin sulfate were added as described for the NNN medium.

Subinoculations from the original cultures were made by transferring a loop of culture medium to the new tube. Cultures were incubated at 15°, 29°, 37°C, and room temperature. The organisms were counted daily with the aid of a hemocytometer. To insure a uniform distribution of organisms, the tubes were shaken prior to transferring a loopful of the medium to the hemocytometer. Only those trypanosomes found in 1 sq mm, which corresponded to 0.1 cu mm of fluid, were counted. Only motile forms were considered.

Culture smears were taken daily from each series. Smears were made by spreading a loopful of liquid on a glass slide. After drying, the smears were fixed in absolute methanol, stained in Giemsa for 20 minutes, rinsed in buffered water, and air-dried. A cover slip was applied, and the trypanosomes were observed microscopically under oil immersion.

RESULTS

Effect of Temperature Using NNN Medium

The number of trypanosomes reported for each temperature is the mean for that series of cultures. The following results were obtained using rabbit serum.

a. Growth at 15°C: Seven tubes were inoculated with T. avium. They did not reach a population peak until the 28th day of growth (Fig. 1). On this day 2178 organisms were present. The initial growth rate was slow. By the 20th day they were approaching their maximum number and continued at this level until the 32nd day.

b. Growth at room temperature (24-28°C): Nine tubes were inoculated. A mean population peak of 1380 organisms was reached on the sixth day (Fig. 2). This level was maintained for two days.

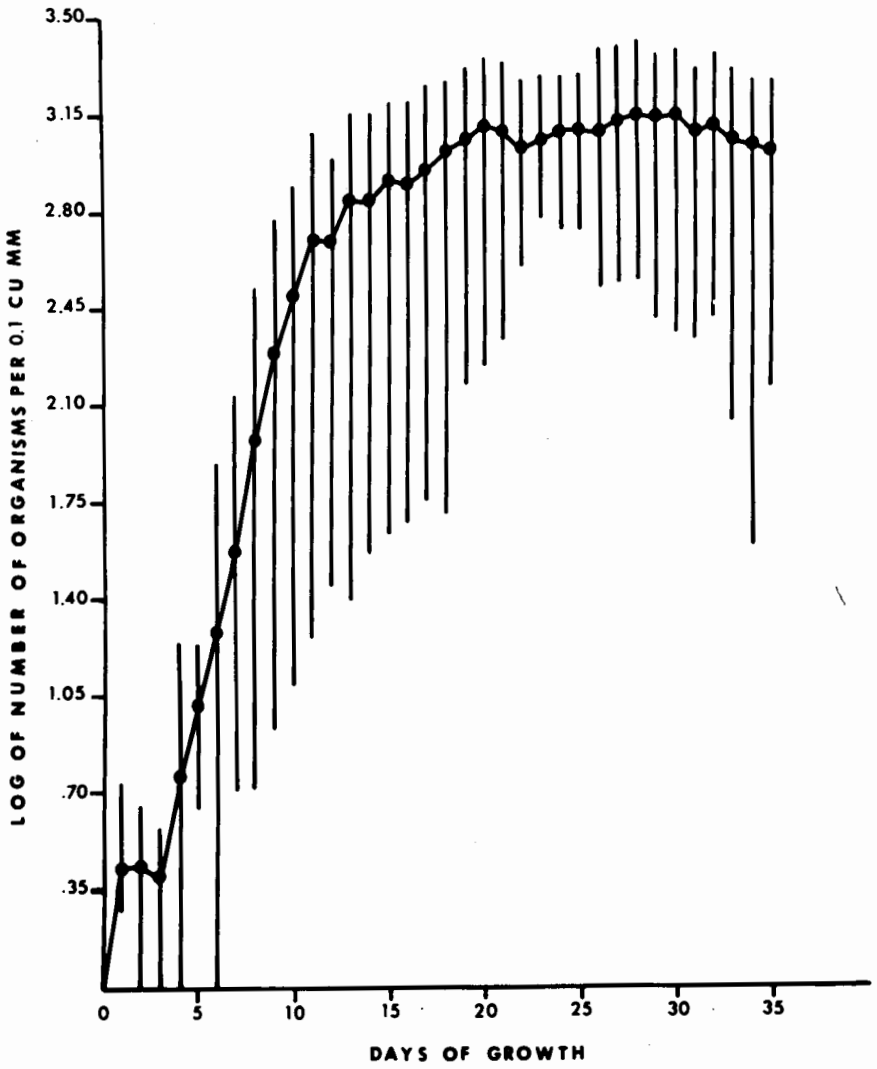
c. Growth at 29°C: Nine tubes were inoculated. Population growth was rapid, reaching a high of 784 organisms on the eighth day (Fig. 3). Though falling the next day, the population number remained near the peak until the 13th day.

d. Growth at 37°C: Four tubes were inoculated, but two cultures died out in a short time. The mean population peak of 788 individuals was reached on the 31st day (Fig. 4). Numbers varied widely throughout the growth period.

NNN medium using sheep serum produced the following results at varied temperatures.

a. Growth at 15°C: Four inoculated cultures used in this study exhibited a slow growth rate. A population high of 781 trypano-

Fig. 1. Growth at 15°C in NNN medium with rabbit serum. In Figs. 1-12 the vertical line represents the range and •——• represents the mean.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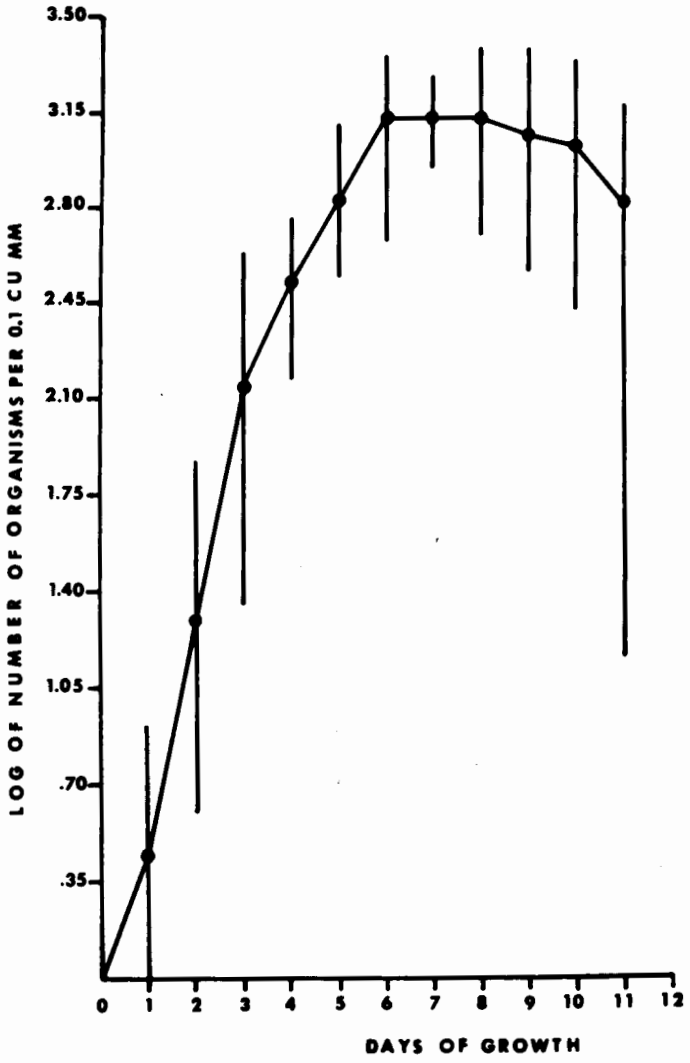
DAYS OF GROWTH

FIGURE 1

Fig. 2. Growth at room temperature in NNN medium with rabbit serum.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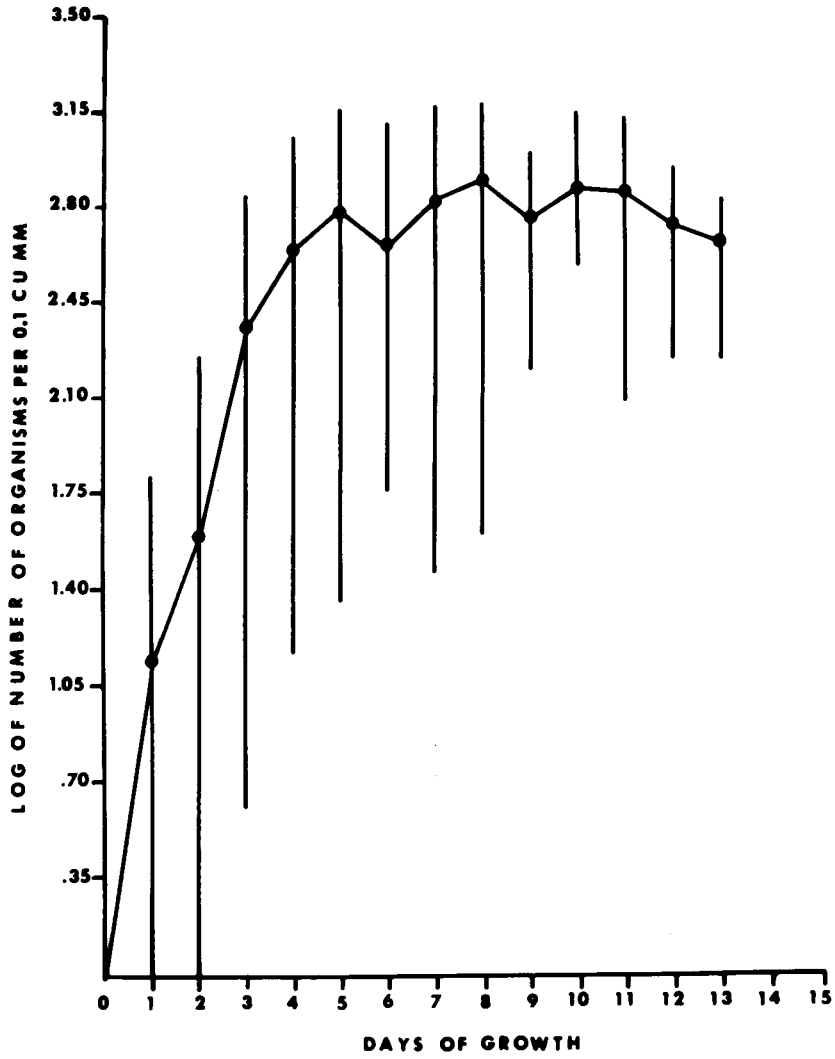
DAYS OF GROWTH

FIGURE 2

Fig. 3. Growth at 29°C in NNN medium with rabbit serum.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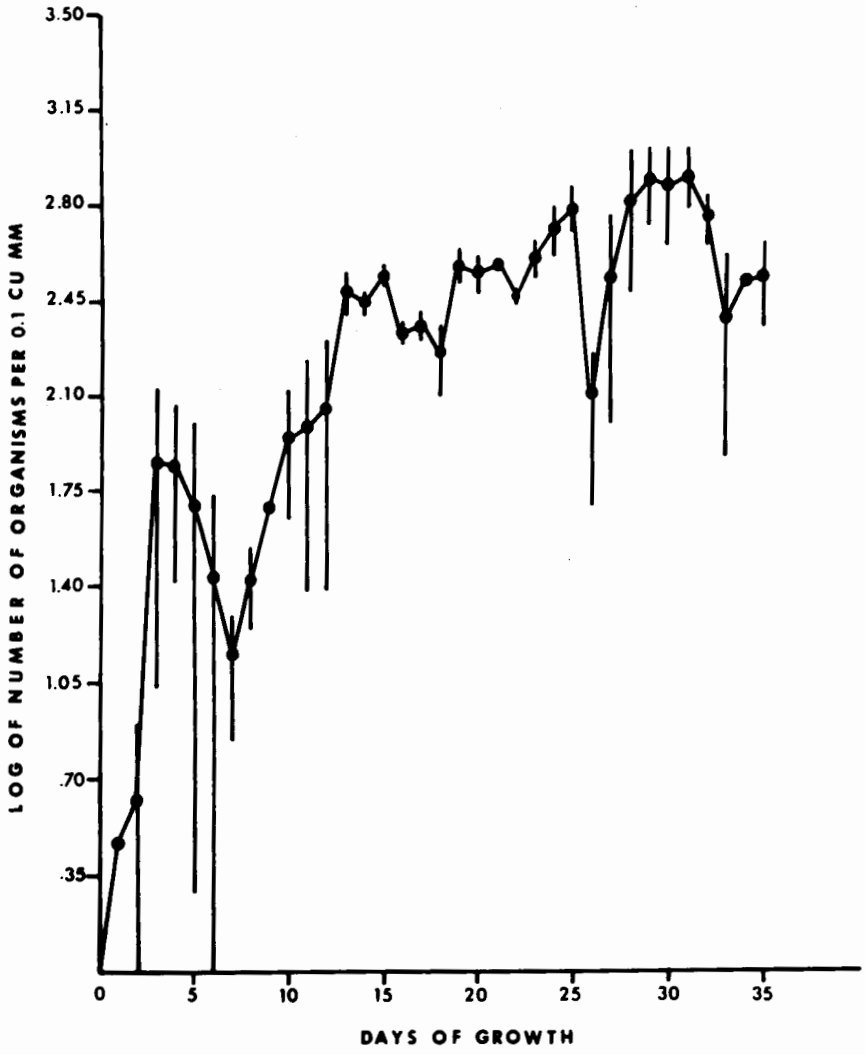
DAYS OF GROWTH

FIGURE 3

Fig. 4. Growth at 37°C in NNN medium with rabbit serum.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DAYS OF GROWTH

FIGURE 4

somes was reached on the 37th day (Fig. 5). They approached this peak on the 31st day and remained at this level until the 39th day.

b. Growth at room temperature (24-28°C): The growth rate of 11 inoculated tubes was rapid, reaching a peak density of 345 on the 13th day (Fig. 6).

c. Growth at 29°C: Five tubes cultured at this temperature reached a mean population peak of 410 organisms on the 16th day following inoculation (Fig. 7). The population remained near this level until the 19th day. The initial growth rate was slow.

d. Growth at 37°C: Five cultures were incubated at this temperature, but none remained viable after the 5th day. The most trypanosomes observed in any culture were three.

Twelve tubes containing NNN medium made with chicken serum were inoculated with robin trypanosomes. Of the twelve, five were incubated at 15°C, five at room temperature, and two at 37°C. The cultures at 37°C and at room temperature died out in a short time. Though the organisms growing at 15°C were viable after 12 days, the highest number observed was 29, on the sixth day; but only six were present on the 12th day.

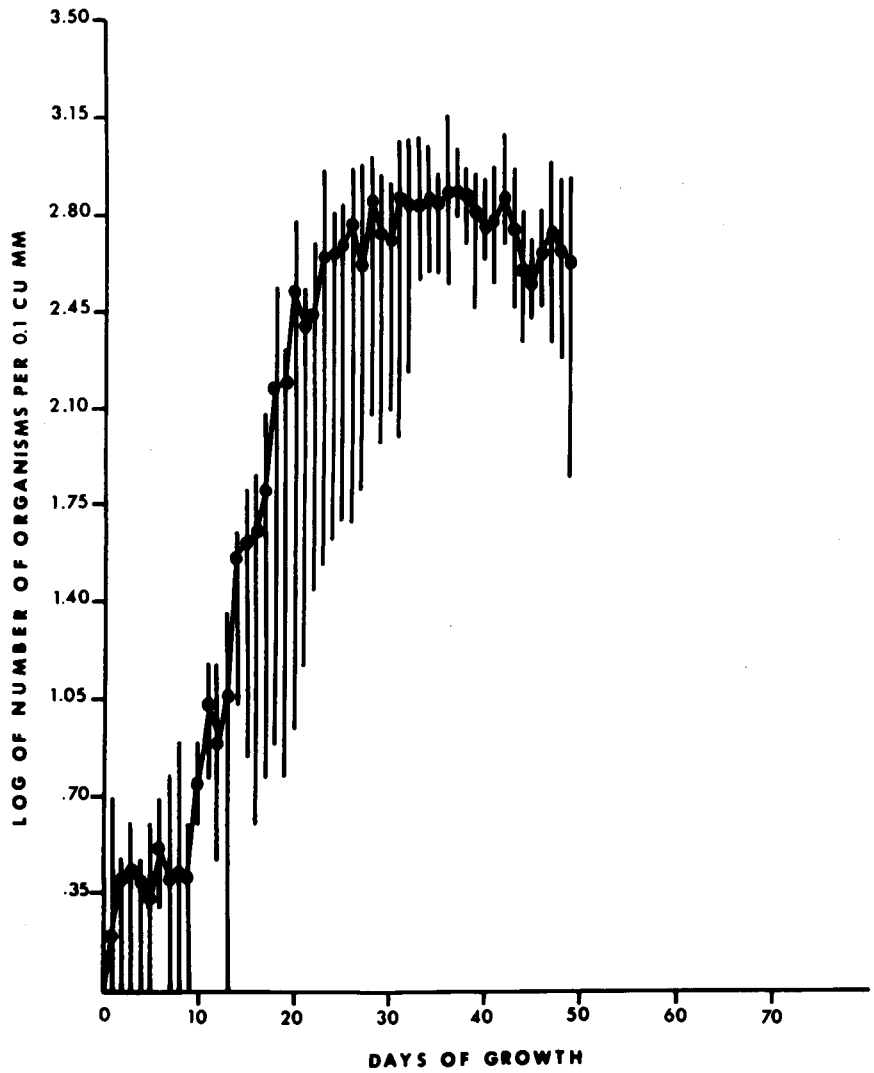
Effect of Temperature Using 4N Medium

Rabbit serum was used in all the 4N cultures.

a. Growth at 15°C: Four tubes inoculated and incubated at this temperature showed a slow growth rate, reaching a population peak of 1550 on the 43rd day (Fig. 8).

b. Growth at room temperature (24-28°C): Five tubes of 4N

Fig. 5. Growth at 15°C in NNN medium with sheep serum.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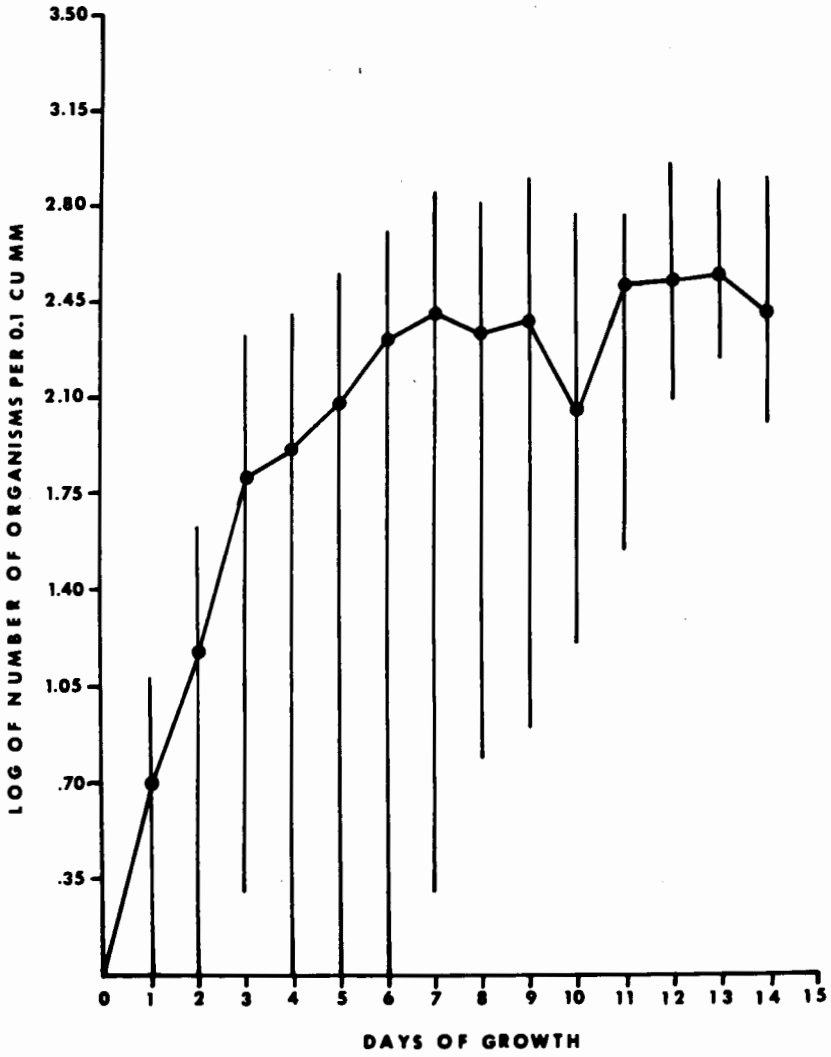
DAYS OF GROWTH

FIGURE 5

Fig. 6. Growth at room temperature in NNN medium with sheep serum.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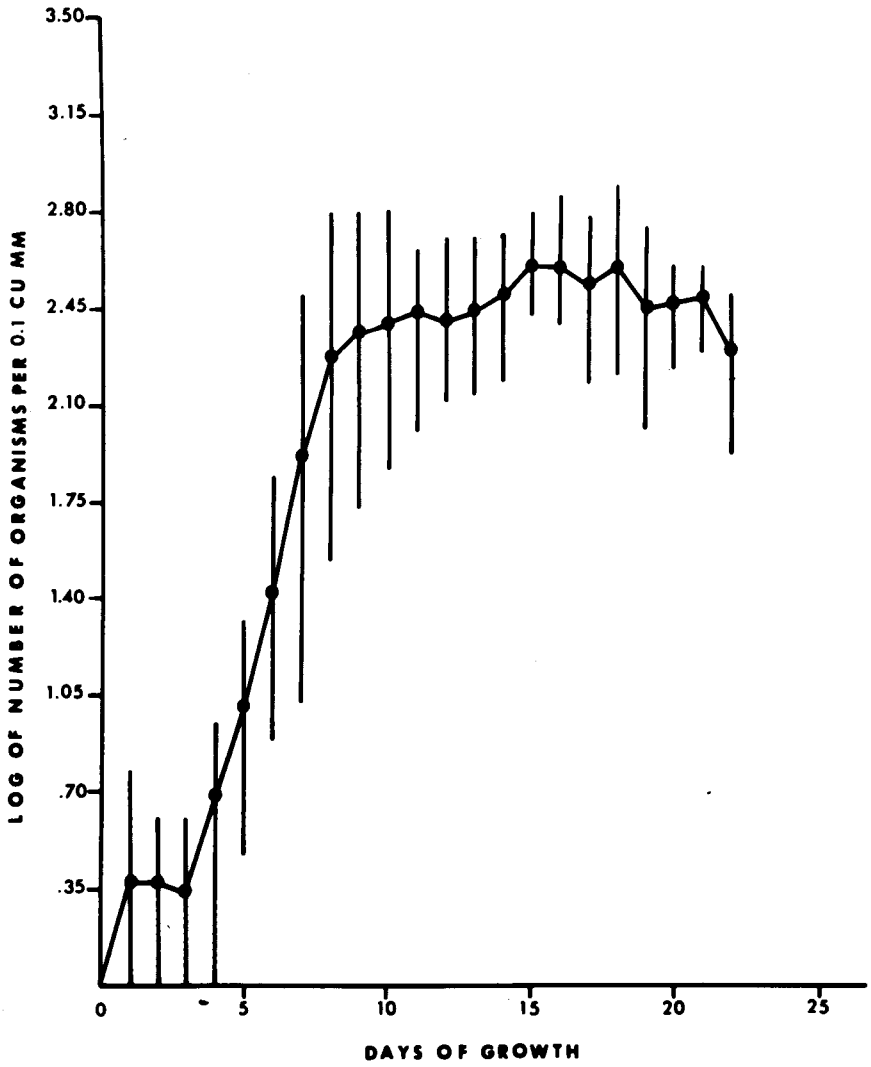
DAYS OF GROWTH

FIGURE 6

Fig. 7. Growth at 29°C in NNN medium with sheep serum.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DAYS OF GROWTH

FIGURE 7

Fig. 8. Growth at 15°C in 4N medium with rabbit serum.

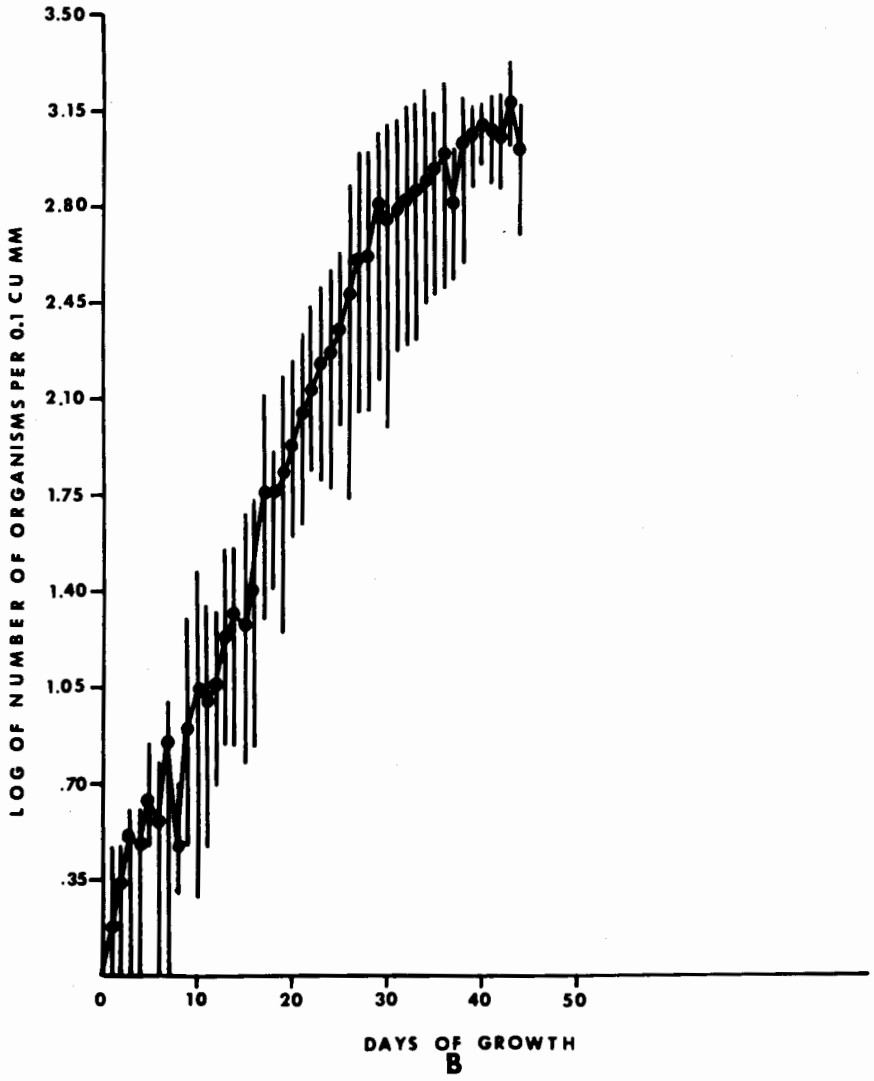
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FIGURE 8

medium inoculated and incubated at room temperature exhibited a rapid growth rate, reaching a population high of 1745 individuals on the 12th day (Fig. 9).

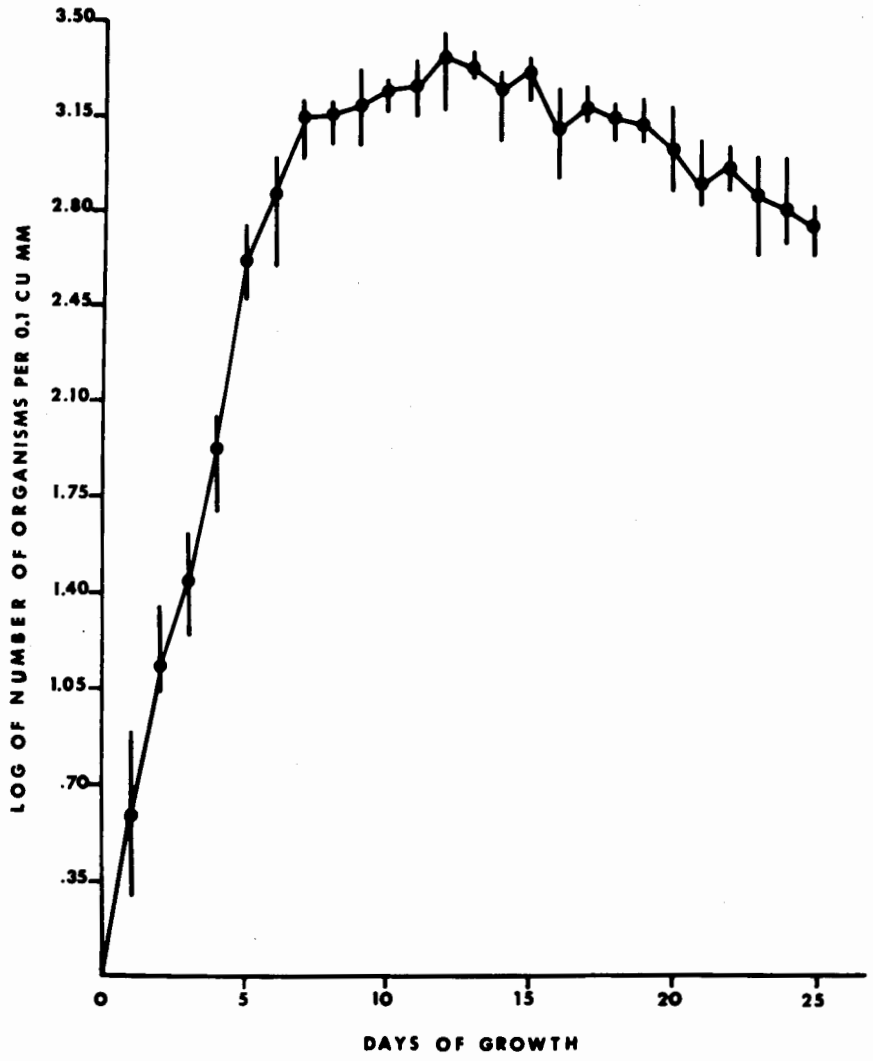
c. Growth at 29°C: Five inoculated tubes cultured at this temperature reached a population peak of 1135 organisms on the 13th day, with the initial growth rate being quite rapid. (Fig. 10).

d. Growth at 37°C: The four tubes incubated at this temperature reached a population high of 414 on the fifth day, but the mean number of trypanosomes was only 12 on the 11th day (Fig. 11). These four cultures were then incubated at room temperature. On the eighth day at this temperature the mean population reached 516 (Fig. 12).

Cyclic Development and Morphology

Daily smears were taken from cultures at the various temperatures. These slides were stained in Giemsa and examined using an oil objective. No cyclic development was detected. The majority of the trypanosomes were slender forms (Fig. 13). Relatively few had a pear-shaped appearance (Fig. 14), and a few were bulbous at one end (Fig. 15). Both slender forms and pear-shaped forms were seen in the process of binary fission (Figs. 16-18). Some forms were undergoing multiple fission, giving the appearance of a rosette (Figs. 16-21). A few leishman bodies were present. Ten per cent of the trypanosomes cultured in NNN medium at room temperature were pear-shaped on the third day. This culture reached a population peak on the sixth day of incubation. This was the highest percentage of pear-shaped organisms observed.

Fig. 9. Growth at room temperature in 4N medium with rabbit serum.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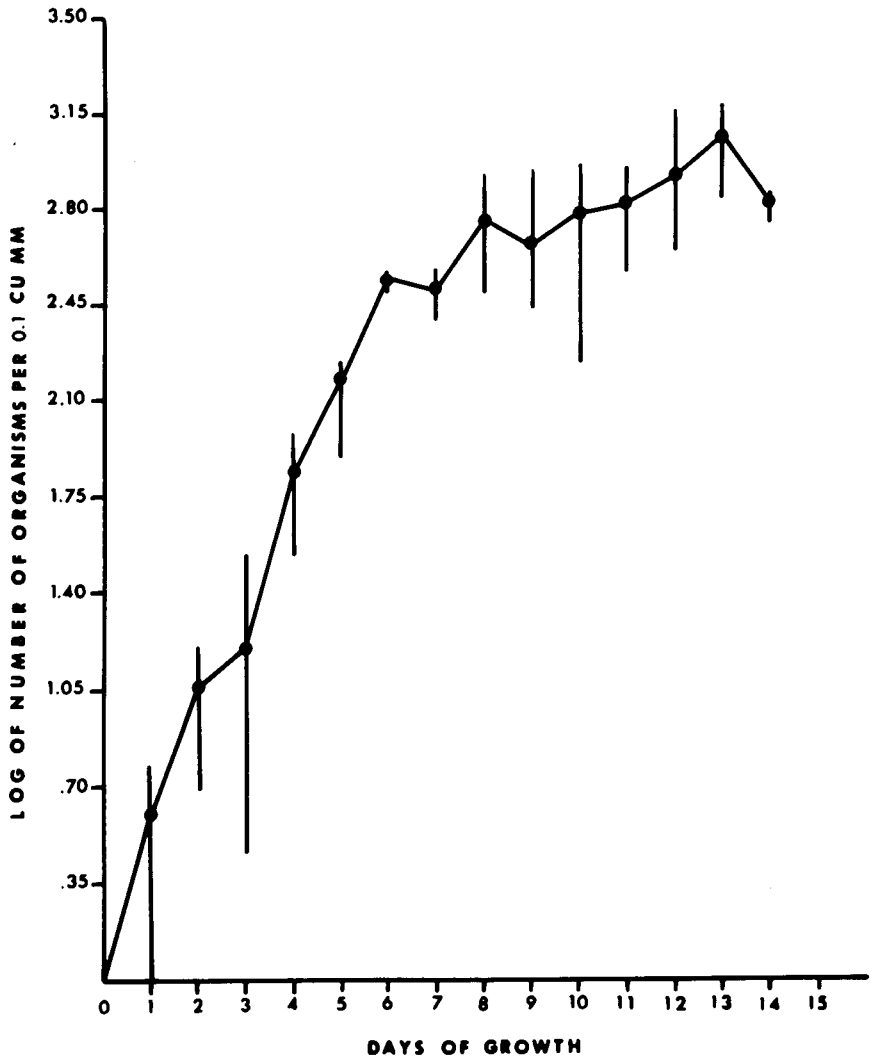
DAYS OF GROWTH

FIGURE 9

Fig. 10. Growth at 29°C in 4N medium with rabbit serum.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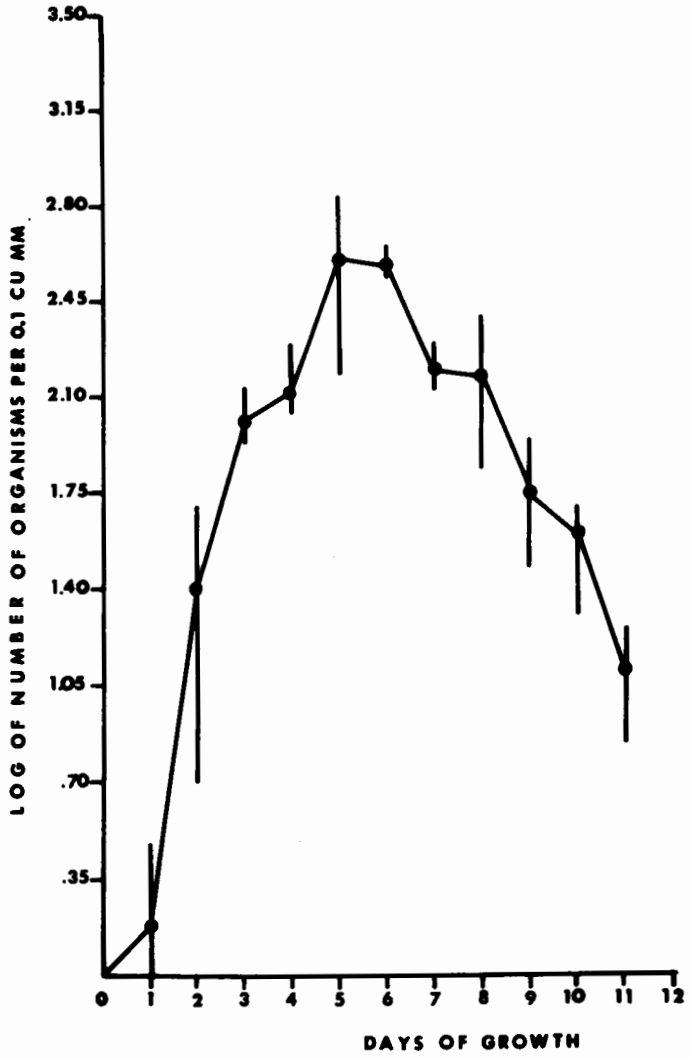
DAYS OF GROWTH

FIGURE 10

Fig. 11. Growth at 37°C in 4N medium with rabbit serum.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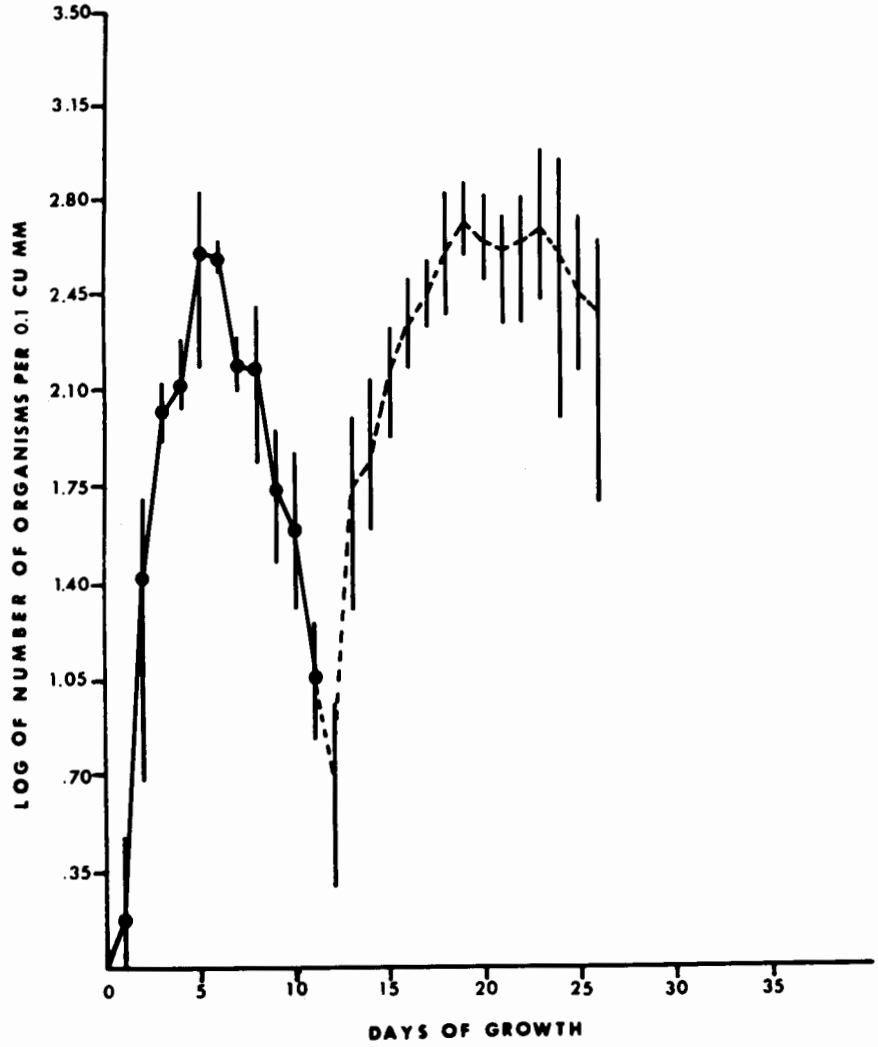
DAYS OF GROWTH

FIGURE 11

Fig. 12. Comparison of growth rates at 37°C followed by incubation at room temperature in 4N medium with rabbit serum. ●—● = growth at 37°C; ---- = growth at room temperature.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DAYS OF GROWTH

FIGURE 12

Figs. 13-21. Photomicrographs of T. avium from a robin (970X).

Fig. 13. Slender forms.

Fig. 14. Pear-shaped forms.

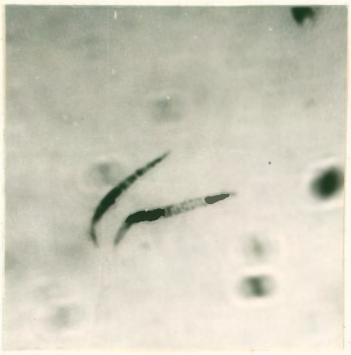
Fig. 15. Bulbous form.

Fig. 16. Early stage of binary fission.

Fig. 17. Late stage of binary fission.

Fig. 18. Near-completion stage of binary fission.

Figs. 19-21. Parasites undergoing multiple fission.



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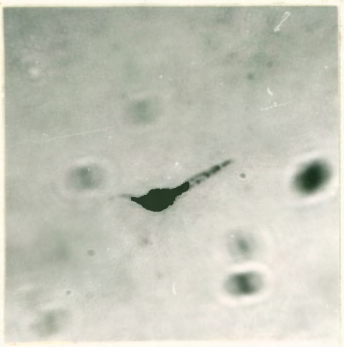

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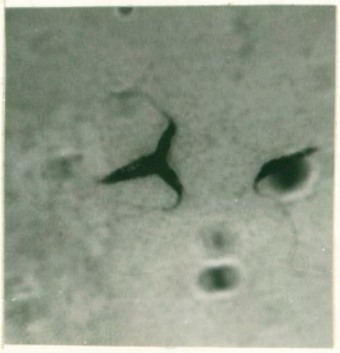

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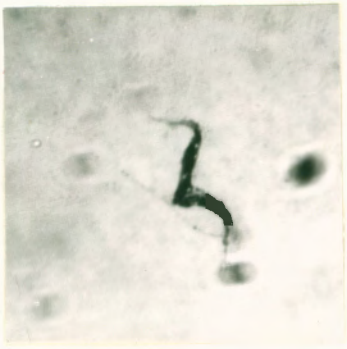

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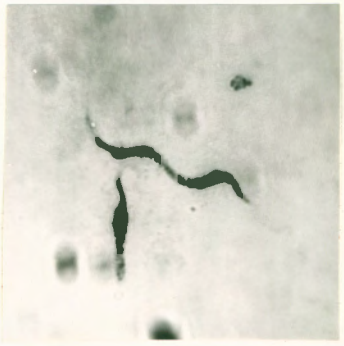

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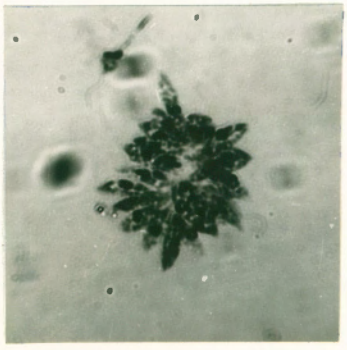

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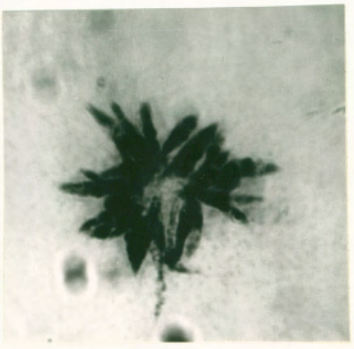

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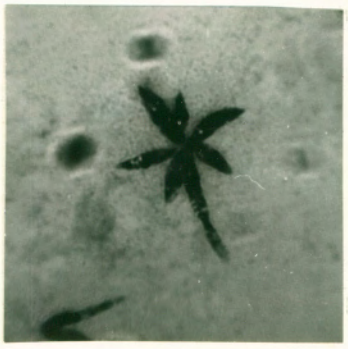


21

The slender trypanosomes were 14.2 (9.9-22.0)μ long and 2.2 (1.1-4.4)μ wide. The nucleus was 2.4 (1.1-3.3)μ long and 1.4 (0.8-4.0)μ wide; it was located 4.1 (2.2-6.6)μ from the anterior end of the body. The oval kinetoplast was located at the anterio-lateral edge of the nucleus.

The pear-shaped forms were 8.1 (4.4-13.2)μ long and 4.6 (3.3-6.6)μ wide. The nucleus was 1.9 (2.2-1.6)μ long and 3.3 (2.0-4.4)μ wide; it was located 2.8 (2.2-3.3)μ from the anterior end of the body. The kinetoplast was located at the edge of the nucleus.

The bulbed-forms were 16.0 (8.8-23.1)μ long and 3.7 (2.2-6.6)μ wide. The kinetoplast was located at the anterio-lateral edge of the nucleus, which was 1.8 (1.1-2.2)μ long and 1.8 (1.1-2.2)μ wide and 2.7 (1.1-3.3)μ from the anterior end of the body.

Many bizarre forms appeared in the cultures which grew at 37°C. They seemed to have undergone partial binary or multiple fission. Many had divided longitudinally for a short distance; then each half had begun to divide longitudinally again. It was as though secondary and tertiary division had proceeded at the expense of the primary division, resulting in a wriggling mass of partially divided cytoplasm.

DISCUSSION

Effect of Temperature

The optimum range for the in vitro growth of T. avium from robins appears to be between 24° and 29°C. When cultured at these temperatures, the trypanosomes reached a population peak in the shortest periods of time (Figs. 22-24). In NNN media with rabbit serum and sheep serum, the highest peaks were achieved at 15°C, but the growth rate was much slower (Figs. 22, 23). In 4N medium the organisms attained the highest peak (1745) at room temperature, but those cultured at 15°C were relatively close (1550), though their growth rate was considerably slower (Fig. 24). The fact that the organisms reached a high population peak after a long period of growth indicates a need for an adjustment period at lower temperatures. After the parasites have become acclimatized, they grow well at this temperature.

Their failure to grow as well at 37°C is perplexing. Those cultured at 37°C in NNN medium with rabbit serum reached a population peak of 788 individuals after 31 days. This peak compares closely with the high of 784 reached in eight days by those trypanosomes cultured at 29°C; however, two of the five cultures incubated at 37°C were not viable. No trypanosomes would grow at 37°C in NNN medium with sheep serum. Those cultured in 4N medium at this temperature reached a high of only 414 individuals, then declined rapidly (Fig. 28). All viable cultures contained a large proportion of bizarre forms when incubated at 37°C.

It is possible that rabbit serum contains some agent which has

Fig. 22. Comparison of growth rates in NNN medium with rabbit serum at various temperatures.

●——● = growth at 15°C; ■——■ = growth at room temperature; - - - - = growth at 29°C;

▲——▲ = growth at 37°C.

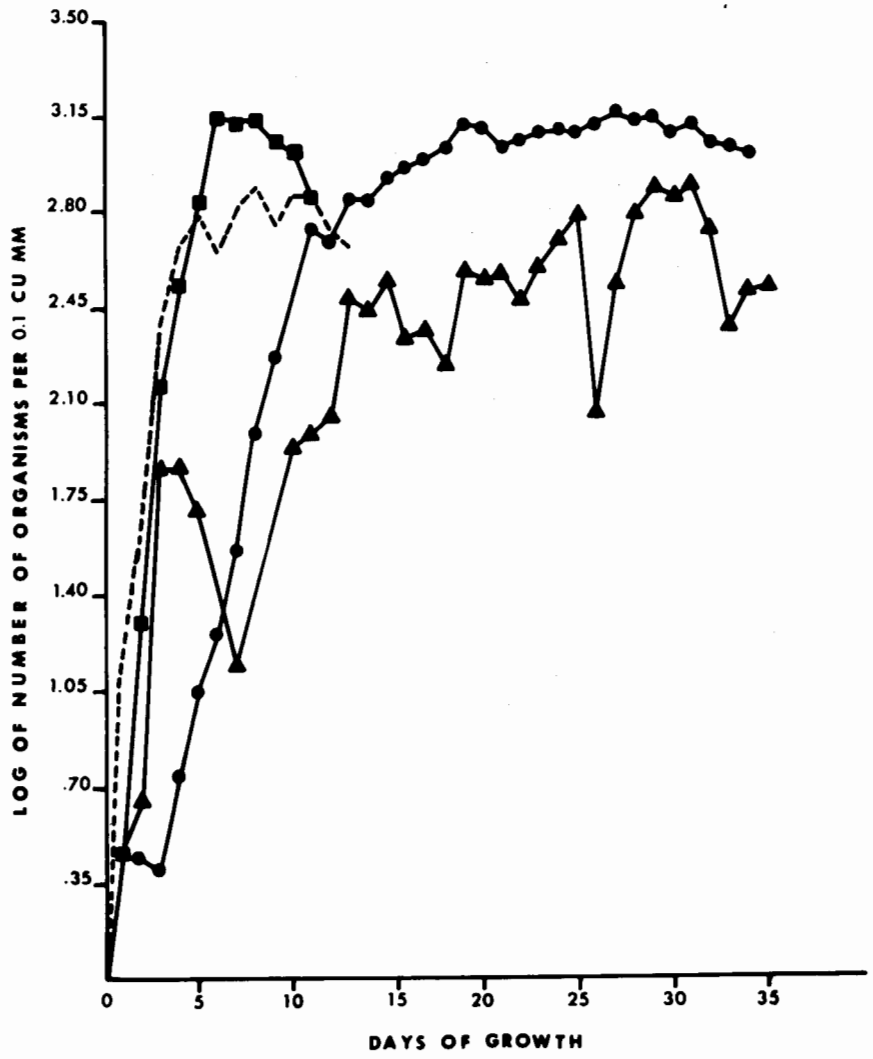
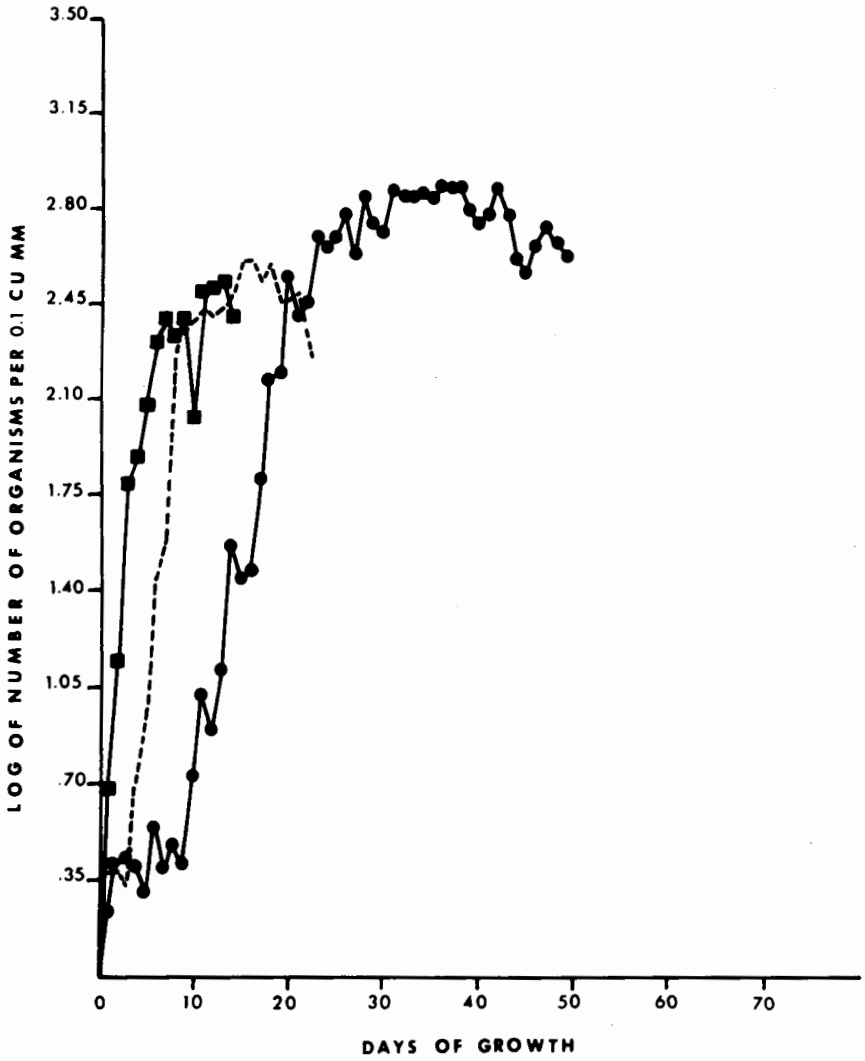

3.50
3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
5
10
15
20
25
30
35
DAYS OF GROWTH

FIGURE 22

Fig. 23. Comparison of growth rates in NNN medium with sheep serum at various temperatures.

●——● = growth at 15 C; ■——■ = growth at room temperature; - - - - = growth at 29°C.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3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
10
20
30
40
50
60
70
DAYS OF GROWTH

FIGURE 23

Fig. 24. Comparison of growth rates in 4N medium with rabbit serum at various temperatures.

●——● = growth at 15°C; ■——■ = growth at room temperature; ---- = growth at 29°C; ▲——▲ = growth at 37°C.

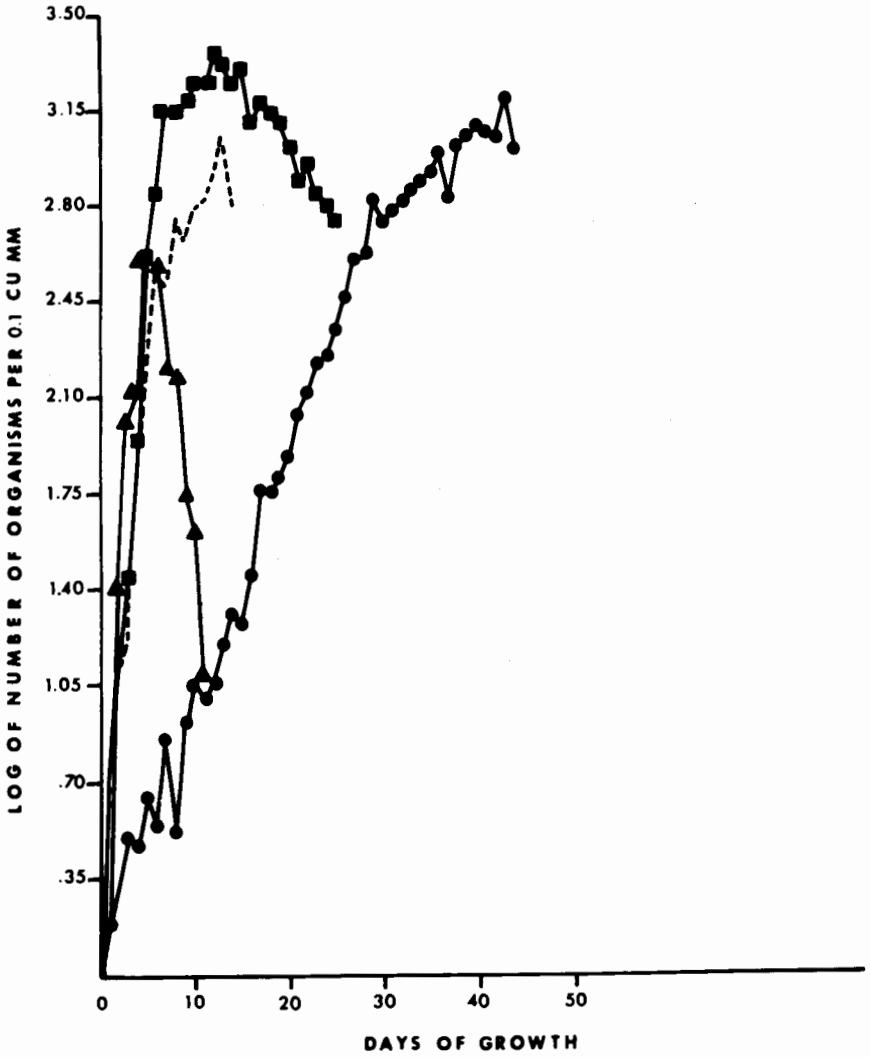

3.50
3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
10
20
30
40
50
DAYS OF GROWTH

FIGURE 24

Fig. 25. Comparison of growth rates at 15°C in different media.

●——● = growth in NNN medium with rabbit serum;

---- = growth in NNN medium with sheep serum;

▲——▲ = growth in 4N medium with rabbit serum.

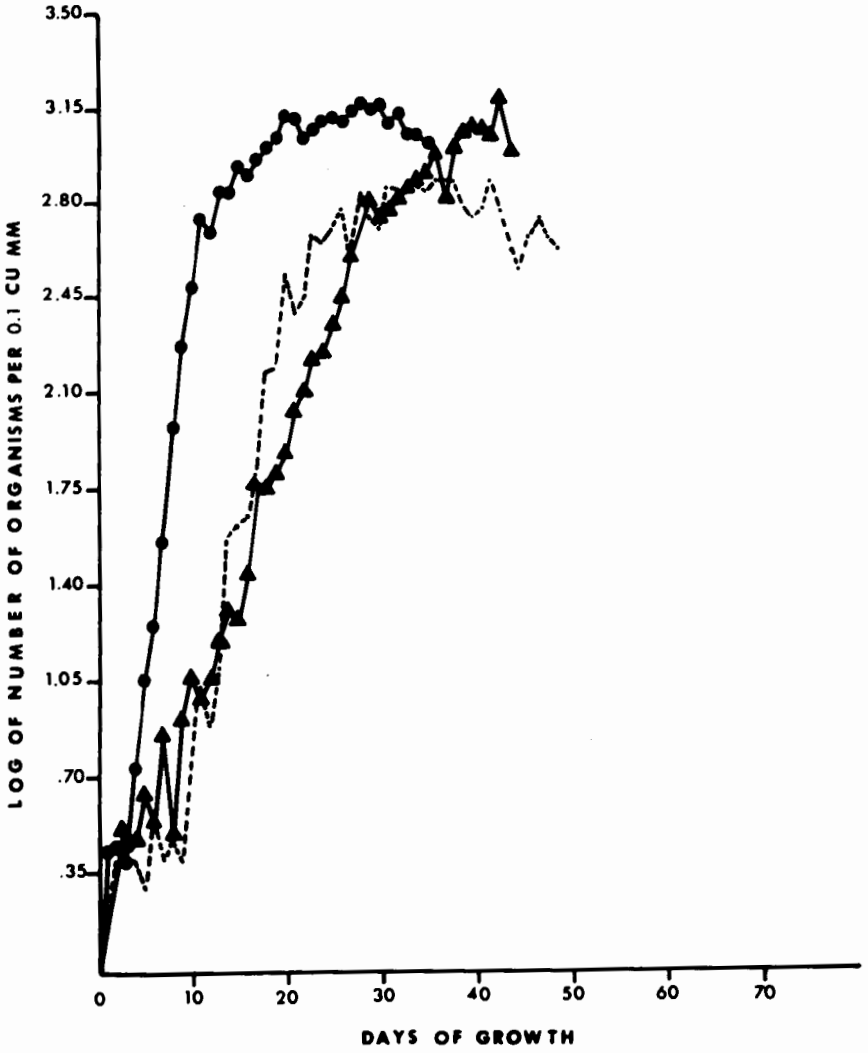

3.50
3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
10
20
30
40
50
60
70
DAYS OF GROWTH

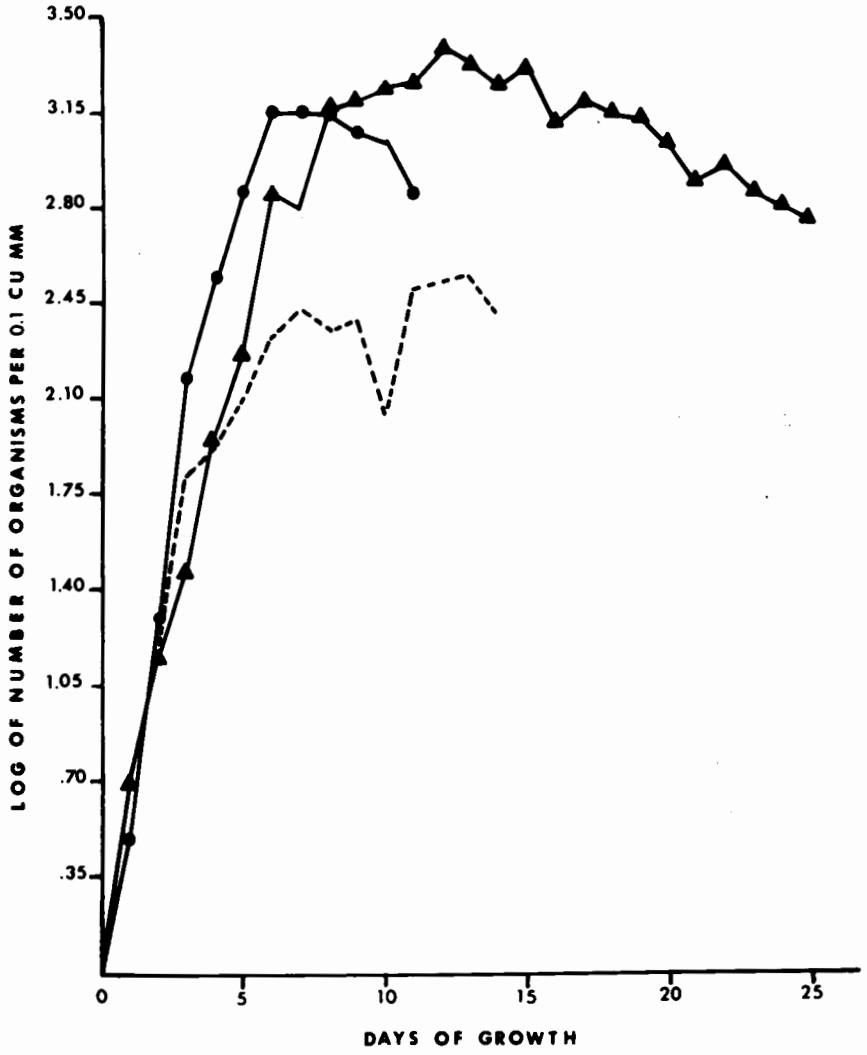
FIGURE 25

Fig. 26. Comparison of growth rates at room temperature in different media.

●——● = growth in NNN medium with rabbit serum;

- - - - = growth in NNN medium with sheep serum;

▲——▲ = growth in 4N medium with rabbit serum.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3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
5
10
15
20
25
DAYS OF GROWTH

FIGURE 26

Fig. 27. Comparison of growth rates at 29°C in different media.

●——● = growth in NNN medium with rabbit serum;

- - - - = growth in NNN medium with sheep serum;

▲——▲ = growth in 4N medium with rabbit serum.

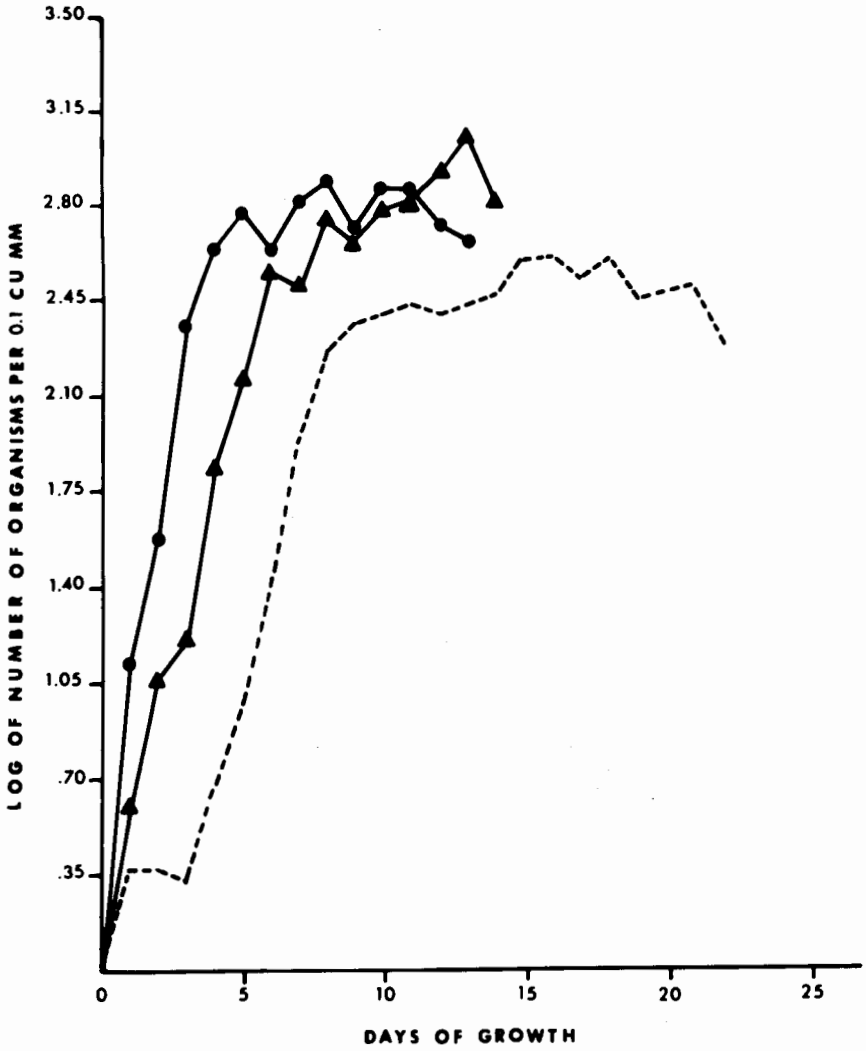
3.50
3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
5
10
15
20
25
DAYS OF GROWTH

FIGURE 27

Fig. 28. Comparison of growth rates at 37°C in different media.

- - - - = NNN medium with rabbit serum;

●——● = 4N medium with rabbit serum.

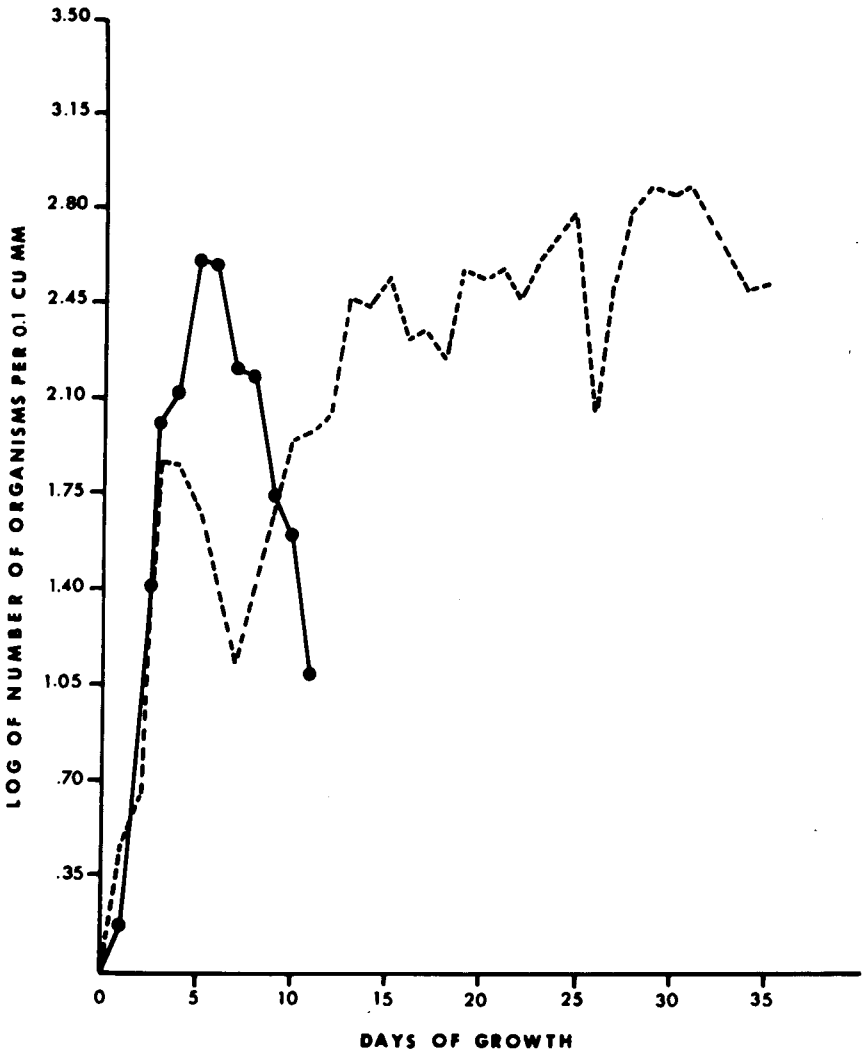
3.50
3.15
2.80
2.45
2.10
1.75
1.40
1.05
.70
.35
LOG OF NUMBER OF ORGANISMS PER 0.1 CU MM
0
5
10
15
20
25
30
35
DAYS OF GROWTH

FIGURE 28

a trypanocidal effect which is inhibited or destroyed at lower temperatures. If this were true, both the sheep serum and chicken serum might be expected to contain a similar defense mechanism. As reported earlier, DeBoe et al. (13) found that serum taken from ducks six months old had a trypanocidal effect.

It is also possible that some nutrient in the serum is used up rapidly at the higher temperature, but this would not account for the failure of the trypanosomes cultured in sheep serum to grow, nor would one expect the organisms to resume a normal growth pattern after being removed from the 37°C incubator and cultured at room temperature.

A more probable hypothesis suggests that the optimum temperature for these organisms in the bird is not the body temperature (40-43°C), but the temperature of bone marrow, in which the incidence of trypanosomiasis is significantly higher. The trypanosomes used in this study were taken from the tibio-tarsus, which has a high surface area to volume ratio; consequently, heat loss would be quite rapid. It would take a relatively large supply of blood to maintain a marrow temperature comparable to the normal body temperature. Equipment necessary for ascertaining marrow temperature of a robin was not available, but it might be expected to be 5°-10°C lower than the body temperature.

Comparison of Media

Though the 4N medium produced higher population peaks in cultures incubated at room temperature and 29°C than those cultured in NNN medium, the growth rates at all temperatures were slower in 4N medium. Sheep serum did not maintain trypanosomes as well as the NNN or 4N.

medium. NNN medium with rabbit serum appeared to be the best medium for culturing T. avium, from the American robin.

Cyclic Development

This investigation did not accord with the results obtained by Lehman (11,12) in his study on cyclic development of culture forms in T. ranarum. Though pear-shaped forms were observed, they did not appear more frequently at any specific time during the growth period. Slender forms were always more numerous.

Much investigation is needed if avian trypanosomes are to be understood more thoroughly. Though they are generally classified as T. avium, it seems unlikely that these organisms found in a variety of birds are indeed the same species. Preliminary observations on magpie, Pica pica, trypanosomes showed forms which did not appear to be like the robin trypanosomes used in this study. More work on the effect of temperature is needed to determine why these parasites do not flourish at 37°C. This could be done by culturing the trypanosomes in tubes of NNN media, each of which lacks a different ingredient. The importance of pathogenic trypanosomes notwithstanding, avian trypanosomes pose many questions of scientific interest.

SUMMARY

1. Trypanosomes from the bone marrow of a robin were cultured at 15°, 29°, 37°C and room temperature in NNN medium with rabbit or sheep sera and 4N medium with rabbit serum. A few additional cultures were made with chicken serum. NNN medium with rabbit serum sustained growth better than the other media. The optimum temperature range for culturing robin trypanosomes appears to be 24-29°C. A temperature of 37° inhibits or prevents growth.

2. Daily smears for each series of temperatures and media were made and stained in Giemsa for morphological studies. The majority of the parasites were slender, though some were pear-shaped or bulbous; a few leishman bodies were found. No cyclic development of the culture forms was detected.

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