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Cultivation Of Trypanosoma Peromysci From Two Species Of Peromyscus

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CULTIVATION OF TRYPANOSOMA PEROMYSCI

FROM TWO SPECIES OF PEROMYSCUS

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CULTIVATION OF *TRYPANOSOMA PEROMYSCI* FROM TWO SPECIES OF *PEROMYSCUS*

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THESIS

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ABSTRACT

Trypanosomes from mice of the species Peromyscus boylii and Peromyscus truei were isolated and cultured in NNN medium and in trypticase soy medium. A continuing culture of trypanosomes developed from blood of mice of the species P. truei. Trypanosomes from the blood of mice of the species P. boylii did not adapt to continuing cultures, but crithidias and rosettes were observed.

The cultures were incubated at temperatures of 1.5 C, 22 C, and 37 C. The organisms incubated at 1.5 C never advanced beyond the crithidial form, but they were observed in the culture medium after 40 days. The organisms were still viable after 23 days at 1.5 C; and one such culture, when placed at 22 C, achieved normal development for that temperature. A temperature of 22 C (room temperature) seemed to be the best temperature for growing T. peromysci. Other investigators have shown this to be true for a number of trypanosomes (T. cruzi, T. lewisi, and others). At a temperature of 37 C T. peromysci trypanosomes existed for at most 6 days in vitro.

In addition to forms described by previous investigators, cystlike bodies were observed in both types of media.

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INTRODUCTION

According to Novy and MacNeal (1904), MacNeal and Novy in 1903 were the first investigators to report a continuing culture of a trypanosome *in vitro*. Some earlier investigators had tried to preserve the organisms in the blood of the host animal, but they were not successful and the organisms died in a few hours. We learn from Novy and MacNeal (1904) that Laveran and Mesnil were able to keep trypanosomes alive at -50 C for 0.5 hr. They were able to keep the organisms at -191 C (temperature of liquid air) for 15 min without loss of viability. This technique is of great value in preserving trypanosomes for long periods. (Polge and Soltys, 1957).

It is fortunate that Novy and MacNeal chose to use *Trypanosoma lewisi* as their first test organisms, as this trypanosome can be cultured more easily than those of other species. After their success with *T. lewisi* they undertook the cultivation of *T. brucei*, using trypanosomes isolated from a dog imported from Africa. The strain was kept alive in mice, and attempts to establish a continuing culture failed with the first 25 mice used. Thanks to their earlier success with *T. lewisi*, the two men were confident that they would succeed, and they did.

From this early work it is evident that species of the genus *Trypanosoma* show variations in their ability to adapt to cultural conditions.

The problem of culturing trypanosomes has been solved many times using several types of artificial environments. The choice of media depends largely on the problem being investigated and the personal bias of the investigator.

Tobie (1964, p. 418) summed up the types of artificial environments

that have been developed by stating "the mammalian trypanosomes can be grown in the following artificial environments: monophasic and diphasic blood agar, dialysate, undefined and partially defined liquid media, semisolid media, avain embryos, and tissue cultures."

It was my intent to isolate the trypanosomes (Figs. 1, 9) from mice of the genus Peromyscus and to establish a continuing culture of these organisms. I chose the traditional NNN medium of Novy and MacNeal for this purpose. I wanted to establish the strain in anticipation of studying nutritional requirements and structure, and I expected to trace the progression of morphological types in vitro by frequent observations of the developing cultures. It was necessary to establish aseptic techniques to insure bacterial-free cultures of trypanosomes. I wanted to avoid the use of antibiotics unless the techniques failed, in which case the control of resulting bacterial contamination by means of antibiotics was to be attempted. It was necessary to determine the optimum temperature for in vitro growth of the organisms in order to obtain maximum concentrations and longevity. In addition, the effect of temperature on progression of morphological stages and the effect of size of the original inoculum were to be determined.

Davis (1952) succeeded in culturing trypanosomes from Peromyscus truei and from Zapus princeps. She was not able to establish a continuing culture of trypanosomes from P. truei, but she did study development of the organisms for the first 14 days in culture. One tube had dead or dying rosettes after 162 days. She did not study these and was not able to transfer them successfully. Her studies of later cultural forms were based on the trypanosomes from Zapus.

I wanted to study development in cultures of T. peromysci for

periods beyond 14 days. To do this I had to avoid excessive transfers and bacterial contamination as Davis attributed the early death of her cultures to frequent subculturing and contamination, as well as to the low concentration of organisms in the original blood samples.

Watson and Hadwen (1914) were the first to report finding a T. lewisi-like organism in a mouse (P. maniculatus) of the genus Peromyscus. In comparing the organism to T. lewisi, they noted a difference in the location of the nucleus and in the shape of the posterior end. In 1914 they described this new organism, which they called Trypanosoma peromysci.

Davis (1952) recognized the weakness of morphological evidence in differentiating these trypanosomes and set about demonstrating this. She reported that only Taliaferro had done work to determine variability of individual trypanosomes. He used T. lewisi.

Davis compared the T. lewisi-like organisms of 12 species of California mammals to each other, to T. duttoni, and to T. lewisi. She found them to be indistinguishable on the basis of morphology. This would seem to shake the system of classification of these trypanosomes. While they cannot be considered separate species on the basis of morphology, as Davis pointed out, there are biological differences and differences in host specificity. She felt that these differences should be considered in classifying the organisms. Using these criteria she considered the trypanosomes of Zapus a distinct species, which she named Trypanosoma zapi.

Establishing cultures of T. lewisi-like organisms for the purpose of studying biological relationships and characterizing species is of great importance. Information obtained from culturing and from studies of

host specificity of the organisms ultimately may provide data for the development of a logical scheme of classification.

METHODS AND MATERIALS

The mice used as sources of trypanosomes were trapped in the Sandia Mountains east of Albuquerque, New Mexico. The traps were set in the lower portions of the Juan Tabo recreation area.

The mice of the area were known to be infected with trypanosomes, thanks to a study by Drummond (1967). I did not attempt to trap a definite species of the genus Peromyscus, but trapping was done in an area where P. boylii was known to be abundant.

The mice were live trapped in Sherman traps baited with oatmeal and peanut butter. In addition to Peromyscus, I caught animals of other genera. Among these were pocket mice (Perognathus), wood rats (Neotoma), and kangaroo rats (Dipodomys). These were all released.

Each mouse of the genus Peromyscus was given a number. This number was used in record keeping, slides were cataloged by this number, and the number was used as a basis for the code numbers on culture tubes.

Captured mice were brought to the laboratory while still in the trap. Then each mouse was shaken from its trap into a plastic bag. This was placed in a wide-mouth etherizing jar so that the open end of the bag was at the bottom of the jar. The lid was placed loosely over the jar and the mouse was allowed to leave the bag as the bag was pulled from the jar.

When I removed the anesthetized mouse from the jar, I used a heparinized syringe with a 22-gauge needle and made a direct heart puncture. As much blood as possible was removed. This operation had to be carried out aseptically in order to avoid bacterial contamination. I tried to prevent contamination by drenching the thoracic region of

each mouse with 70% ethyl alcohol, but the blood was still contaminated when introduced in culture medium. So I found it necessary to remove the skin from a small area of the thoracic region of each mouse and to apply alcohol to the subdermal tissue before making the heart puncture.

Once the blood had been removed, the mouse was sacrificed in the ether jar, and the body was weighed and sexed. Each mouse was identified by a tag attached to the right hind leg, and then the carcass was frozen for later identification to species.

The blood in the syringe was used to prepare a hanging-drop slide and a permanently stained slide. Both of these slides were examined for the presence of trypanosomes. When I began the study, I had intended to attempt culturing the organisms from the blood of every mouse captured. As this was found very time consuming and required an excessive amount of glassware and media, I decided to inoculate only blood found to be positive by slide examination.

The permanent slides were made in the form of blood smears. Blood smears from the first mouse to those of the 14th mouse were stained with Wright's-Giemsa stain (Fuscillo modification). The stain worked well, but a great deal of background debris was stained. Wright's stain was used for the remainder of the slides because it did not stain the background material so much. However, the organisms did not take up the stain so well either.

The remaining blood was inoculated into tubes of media. The number of tubes used depended on the amount of blood available. Approximately 5 drops of blood were placed in each tube. I added 0.5 ml to some tubes to determine the effect of inoculum size on development of the cultures.

I used NNN medium for isolation of the organisms. This is the medium that MacNeal and Novy used to grow T. lewisi and T. brucei and this was the medium chosen by Davis (1952) for growing T. peromysci and T. zapi. I followed her instructions for preparation, using 900 ml of distilled water, 14 g of agar, and 6 g of sodium chloride. The mixture was brought to the boiling point to dissolve the agar and was then filtered, distributed in 6-ml portions into test tubes, and autoclaved. I used screw cap culture tubes so that evaporation could be held at a minimum. One ml of sterile defibrinated rabbit blood was added to the melted agar in each tube. The tubes were then slanted and allowed to cool.

I also used a modification of this medium. According to Davis (1952), the modification was devised by Mathis in 1906. In this process NNN medium is cooked for 3 hr at 70-80 C.

On occasion I substituted sheep blood for rabbit blood in making the NNN medium. This will be pointed out in discussing results.

Physiological saline was added because only a minimum of water of condensation appeared in the tubes. The organisms grow in the fluid above the solid medium. Evaporation became a problem and at times as much as 5 ml of saline was added; usually 0.5 ml of saline was sufficient.

In one series of tubes, I used trypticase soy broth as the base for culture medium. I added only the blood of the mouse to this in attempting the initial isolation. This medium was used by Iralu (1967) for culturing T. cruzi. When transfers were to be made, I used a blood lysate to supply necessary nutritional factors. I did not use hemin and serum as did Iralu, nor did I use whole blood (Simpson, 1959).

When it appeared desirable to use a medium free of cells, the blood

lysate used was prepared by adding 10 ml of chilled, distilled water to 1 ml of heparinized rabbit blood. The resulting fluid was filtered through a Seitz filter. This sterile fluid was then added to each tube in the amount of 10% of the total volume of fluid in the tube.

On one or two occassions I found it necessary to add penicillin in order to free the culture of bacteria. I added Terramycin to other cultures, but this was of little value.

Attempts were made to cultivate organisms at three different temperatures. The tubes at 37 C were kept in an incubator. The second set of tubes was kept at room temperature in an area with thermostatically controlled heat with the temperature set for 22 C. The third set of tubes was stored in a refrigerator at 1.5 C.

Examinations of cultures were made using hanging-drop slides, dark-field techniques, and stained slides. The stained slides were not very valuable, although the stains used were the same as those employed to stain the original blood smears. In an effort to discover why the slides were so poor, I followed the steps in each process with an examination of the slides. However I was not able to determine the source of my difficulties.

Cultures were examined at varying intervals. I felt it advantageous to leave several tubes sealed for some time. Iralu (1967) mentioned that trypticase soy broth cultures of T. cruzi were delayed in development or even destroyed by opening them too soon.

It was necessary to set aside certain tubes for observation at a later date. It was also necessary to open some tubes immediately to observe the early morphological changes occurring in the organisms.

All tubes were checked once a month to determine if organisms could be observed. This was necessary because certain of the cultures

would appear negative for a time before the trypanosomes were seen once more.

In the course of determining temperature relationships, I wanted to see if the organisms were still active at low temperatures. I used an inoculating loop to transfer culture material on to hanging-drop slides. The loop had to be heated to prevent bacterial contamination. When the loop was introduced into the cooler medium, it no doubt raised the temperature of the medium. Once the slide had been prepared, the higher temperature of the room would affect the temperature of the slide. The heat produced by the substage lamp was considerable. To prevent temperature increase, I placed a mirror on the microscope, prepared a slide and attached it to the microscope stage, and then placed all of these in a walk-in-refrigerator. I allowed the system to cool for 30 minutes. The temperature of the stage was reduced to 6-8 C.

RESULTS

I succeeded in keeping trypanosomes from Peromyscus boylii and Peromyscus truei alive in culture, but the organisms from P. boylii did not become adapted to a continuing culture as did the trypanosomes of P. truei. The trypanosomes from P. boylii did remain alive long enough to develop dividing forms (Fig. 2), and rosettes of four or five elongate organisms were seen (Fig 3). The original NNN medium seemed to work well in keeping the organisms alive. Although the organisms remained viable on Mathis variety of NNN medium, rosettes never developed. Water loss seemed to be the major problem encountered in this series of cultures.

I used 13 mice before I obtained a successful series of cultures (Table 1). Of the 13, only two mice were shown to be infected by means of microscopic examination of blood at the time of its removal from the mice. Blood from both of these mice was inoculated into NNN medium. Tubes with blood from mouse 5 were incubated at 37 C, and it was not surprising that organisms did not develop. Organisms from mouse 11 were overgrown by bacteria.

Mouse 14, the source of the first successful series (Table 2), was more heavily infected than any of the other mice examined. I removed the mouse from the trap on a very cold morning. It seemed to be almost frozen.

The trypanosomes from mouse 15 were able to persist for a short time at room temperature (Table 3). One tube was contaminated when improperly sterilized saline was added. Transfers were made from this tube and were refrigerated. The parent culture was overgrown by bacteria by the following day. The subcultures were doing well in the

refrigerator and the bacteria were inhibited by the lowered temperature.

The trypanosomes were very active after 1 day at the lower temperature. As the number of days increased and the cultures became older, the organisms became more and more sluggish. They became more slender as well. Some of them did survive for a period of 40 days. The companion tube at 22 C had organisms in it for only 15 days.

Mouse 16 (P. truei) supplied the trypanosomes for the first successful cultures that I obtained from this host species (Table 3). One of the tubes from this series was maintained for a period of 110 days.

One of the tubes of the 16 series survived for 6 days at 37 C. Very peculiar club-shaped forms (Fig. 4) were present and were gone by the seventh day. No other culture lasted longer than 48 hr at the increased temperature.

Perhaps the most successful set of cultures (Table 4) was initiated from the blood of mouse 20, a specimen of P. truei. A whole series of cultures and subcultures was established from this group of organisms. The tubes containing trypticase soy broth were the successful ones.

I noted that in tube 16-5 a long period elapsed when no organisms could be found. Then they reappeared. I was curious to know why this occurred and I felt that the only way to find out was to use a fluid medium. Using this medium, I was able to identify the cystlike bodies (Figs. 6, 10) referred to by Iralu (1964) and by Deane and Milder (1966).

In addition, I was able to observe the reoccurrence of forms with partially complete undulating membranes (Fig. 7) long after such forms had disappeared from both the NNN and TSB cultures.

One of the TSB tubes was kept at 22 C; the other was kept at 1.5 C.

This group of organisms seemed to be much more motile than were earlier ones. Almost at once I noted rapidly swimming flagellated and very slender leptomonadlike forms in addition to the slower moving organisms with the undulating membranes.

The tubes kept in the refrigerator appeared to be negative after 8 days. I examined them with a microscope kept in a walk-in refrigerator. The temperature was between 6 and 8 C. Dividing forms were observed. The organisms were motile, but sluggish.

By the eighth day, the cultures were negative. I removed culture 20-6 from the refrigerator and placed it at 22 C. A few organisms were seen the following day. These disappeared, and no sign of life was evident for almost a month. Then rosettes were observed, cystlike bodies were formed, and a few swimming individuals were seen.

The apparent absence of organisms from tube 20-7 was of shorter duration. The culture remained positive until some blood lysate was added. This seemed to cause some type of an agglutination reaction. Two days after this addition, a metacyclic trypanosome was seen.

The progression of morphological forms in tube 20-7 followed the pattern described by Davis (1952). Trypanosomal forms persisted for about a week. Crithidia developed from these and replaced both the trypanosomal forms and the rapidly motile, flagellated forms.

After 23 days in the culture, small oval individuals were seen. These were in large masses and were flagellated (Fig. 5). Cysts appeared shortly after this time. As I already mentioned, a metacyclic trypanosome was seen in the culture on about the 80th day.

DISCUSSION

Trypanosomes can be grown on a wide variety of media. I chose to use the traditional NNN agar because it had been used by Davis (1952) with some success. Once I had managed to keep organisms alive for some time on this type of medium, I decided to try using an entirely fluid medium. I hoped that this would allow me to see more stages of the developing trypanosomes. This proved to be the case. I chose trypticase soy broth medium because it seemed to be a convenient form. Other types of media might have been used. I might have used the "LIT" medium of Deane and Milder (1966) or the SNB-9 medium of Diamond and Herman (1954), but I thought that using one medium instead of another medium would make little difference. Tobie (1964) pointed out that almost everyone who uses blood agar varies it in some small way.

I used penicillin to destroy bacterial contamination in certain tubes, but it was not used as a general prophylactic because I wished to avoid adding unnecessary substances to the medium. The penicillin worked well in most cases, but in one set of tubes it failed. The contaminant was a highly motile bacillus introduced into the culture when a 2% suspension of sheep erythrocytes was added.

I tried other drugs, Terramycin for example, but these did not work well.

Seneca, Henderson, and Harvey (1949) reported using penicillin and streptomycin to rid hemoflagellate cultures of bacteria.

In at least one way, the trypanosomes of mice of the genus Peromyscus resemble T. brucei, the trypanosome causing nagana. Both are difficult to culture. Novy and MacNeal (1904) faced some problems in getting T. brucei to grow in vitro. They determined that sustained

growth was best at room temperature (25 C). They found increased growth to occur at 34 C, but the cultures did not last so long.

Similiar temperature relationships seemed to exist for T. peromysci. The organisms remained viable in vitro at 37 C for only 6 days at the most.

Greenblatt and Glaser (1965) found that Leishmania enriettii would not grow at temperatures above 37 C. At temperatures above 30 C there was decreased viability in spite of initially elevated respiratory capacity. Between 35 and 37 C they noted marked morphological changes, including loss of flagella and formation of inclusion bodies which they felt to be lipid in nature. Greenblatt and Glaser (1965) attributed the loss of viability to an increased cell permeability. Between 10 and 40 C there was a 30-fold increase in permeability, but there was only a twofold increase in the respiratory rate and no lysis occurred.

These findings would account for the occurrence of Leishmania organisms in cooler regions of the body. This is not true of trypanosomes, as they are in the circulating blood. This might lead one to believe that the suggested temperature relationship does not exist for trypanosomes.

There is some evidence to dispute this. Amrein (1957) studied the effect of environmental temperature on Trypanosoma cruzi. He found that at 35 C no mice died and the infections disappeared. If the mice were kept at 10 C, the infections were 100% fatal. In some way, the temperature of the environment affected the organisms, even within host tissues. Administration of adrenocortical hormone reversed the situation.

It would seem that substances in the blood are capable of supplying the necessary materials for life at a faster rate than the rate of

leakage of vital material. This would not be the case in culture.

Some investigators attributed morphological changes of trypanosomes to temperature. Herbert (1965) mentioned that Trautmann in 1922 reported bringing about the change from cultural form to trypanosomal form by incubating cultures at 35 C. Trejos, Godoy, Greenblatt, and Cedillos (1963) grew T. cruzi in cultures of "L" cells (L 929, Microbiological Associates). They incubated these cultures of mouse fibroblasts at several temperatures and from the results they hypothesized that temperature alone was responsible for morphological differences observed.

Steinert and Boné (1956) attributed certain morphological changes in cultures of T. mega to addition of serum. The trypanosomal forms appeared in the cultures after the addition of serum. I noticed a similar occurrence when I added serum to tube 20-7. I was surprised to observe a form with a complete undulating membrane months after such forms had apparently disappeared from the culture.

The continued existence of the trypanosomes at a temperature as low as 1.5 C is not surprising, because Laveran and Mesnil (as reported by Novy and MacNeal, 1904) found that trypanosomes survived for 15 min at temperatures as low as -191 C.

The trypanosomes in cultures incubated at 1.5 C were very active for the first 2 or 3 days. As time passed, they became less active and at the same time more slender.

When the organisms were examined at 6 to 8 C, they were slightly motile. The one trypanosome that I saw dividing had probably initiated the process while the slide it was on was at room temperature.

I believe that the metabolic rate of the organisms was slowed down at the lower temperature and that the organisms eventually wasted away.

This degeneration was reversible after 23 days as the culture removed from the refrigerator at this point did develop.

The degeneration that appeared to be occurring has been prevented by freezing the organisms very quickly. A technique for doing this was suggested by Polge and Soltys (1957). This technique can be used to preserve trypanosomes for indefinite periods of time.

Room temperature (22 C) seemed the best temperature at which to cultivate these organisms. At this temperature the organisms persisted for longer periods of time, reproduced, and passed through the various morphological stages described by Davis (1952). The later stages in development of T. peromysci resemble those of T. zapi.

The earliest forms seen were the trypanosomal forms. These gave way to transitional forms. In a week or 10 days, crithidial forms developed. After a period of 20 days, the presence of rosettes was noted. These rosettes were composed of 5 to 15 or 20 individuals. The individuals themselves were long and slender. Larger oval forms took the place of these in a short time. With the passage of time, the rosettes became larger and the component individuals smaller. Leptomonad and leishmanial forms were observed. I did not observe metacyclic forms until the cultures were at least 80 days old. These forms may well have occurred prior to this, but the small numbers of such forms may have prevented their detection.

The rosettes were, in older cultures, combined to form masses of several hundred organisms. These organisms were very small, but many still had flagella. Occasionally one or two organisms could be seen breaking away from the rosettes. Often they were attached by the flagellum to the central mass of the rosette.

Davis (1952) referred to the masses of rosettes. She felt that the masses showed several growing centers and that different types of individuals could be seen at levels in the rosettes.

In addition to the forms reported by Davis, I found what I believed to be the cystlike bodies of Deane and Milder (1966). They noted the structures in "LIT" liquid medium cultures of T. conorhini. The cysts were noted initially to have the appearance of two or more large crithidias fused together. I did not observe this phase although I did find some very small cystlike bodies. These were not much larger than one or two individuals. I did not observe all of the steps in formation described by Deane and Milder, but I did see a variety of sizes of cystlike bodies. I observed the stalklike structures attached to some of the cystlike bodies and I did see organisms attached to these bodies (Fig. 6). In one instance, I saw an individual half in and half out of a cyst.

I was convinced that there was some type of growth activity occurring in the cystlike bodies when I observed two mature structures side by side (Fig. 8). One had ruptured, and its contents were spilled out. The other was still intact, and I could see organisms within it. I observed these structures for some time, and then preserved them in the refrigerator. After 6 hr I found that the second structure had released its organisms. I did not see any flagellated forms in the masses of individuals.

Iralu (1964) induced cyst formation in cultures of T. cruzi by adding urease to the medium. Deane and Milder (1966) added nothing extra to get cyst formation in SNB-9 medium. Iralu used TSB medium for growing cysts. I did the same, but I did see some cysts in the tubes of NNN

medium. They did not appear to be so numerous there.

I am not aware of the function of the cystlike bodies. Deane and Milder hesitated to call them cysts because they do not have a true cyst wall. They were not much more resistant to such environmental stresses as heat, drying, and hypotonic solutions than were other stages according to Deane and Milder. They considered the bodies possibly involved in some type of transfer of genetic material.

Once the cystlike bodies had released their organisms, the bodies continued to float about as hollow, saclike structures.

In the summer of 1967, I attempted to capture infected Peromyscus. I was not very successful. I began in June with preliminary trapping, and I caught four mice that month. None of them appeared to be infected. In September, I caught an infected mouse. This was the first infected mouse of the 10 captured to that time. It was the middle of November before I caught another infected mouse. I had no more problems from that point up until January when I quit trapping. I expected this having read information presented by Drummond (1967). He stated that a seasonal variation in the percentage of infected mice existed. The peak periods of infection are in December and in May.

The trypanosomes of Peromyscus do not adapt to culture with the ease that T. lewisi does. Even when cultures of T. peromysci become established, the concentration of organisms is very low. Such cultures are not of much value for studying biological relationships.

In some way, the concentration of the organisms in vitro will have to be increased in order that the cultures be of value for further research. This might be accomplished by concentrating the organisms in the original inoculum. Taliaferro (1932) used such a method in growing

T. lewisi in vitro. He centrifuged the blood sample to be used and removed excess serum.

SUMMARY

1. During the period from September 1967 to January 1968, 20 mice of the genus Peromyscus were trapped in the Sandia Mountains east of Albuquerque, New Mexico.
2. Hanging-drop slides and slides stained with Wright's-Giemsa stain (Fuscillo modification) or Wright's stain were prepared from the blood of these mice.
3. Blood containing trypanosomes was inoculated into NNN medium or NNN medium and trypticase soy broth medium. The cultures were incubated at temperatures of 1.5 C, 22 C, and 37 C. Examination of the cultures was made using hanging-drop slides, stained slides, and dark-field microscopy.
4. Penicillin was used in cultures that were contaminated with bacteria. Bacteria quickly overgrow these trypanosomes in culture unless some action is taken to prevent this.
5. Trypanosomes from mice of the species P. truei and P. boylii were kept alive in culture. The trypanosomes from mice of the species P. boylii were unable to adapt to continuing culture, but rosettes of elongate organisms were observed. The organisms in the blood of mice of the species P. truei did continue to exist and develop in the cultures.
6. Crithidial, leishmanial, leptomonad, and metacyclic forms were observed in cultures incubated at 22 C. Organisms in cultures incubated at 37 C survived for 6 days at the most and were club-shaped in form. Organisms in cultures kept at 1.5 C were prevented from undergoing major morphological changes. They did remain viable for 23 days. Only trypanosomes and crithidia were observed from

these cultures.

7. Cystlike bodies were observed in both NNN and trypticase soy broth media, but more were observed in the trypticase soy medium than in the NNN medium. Other investigators have reported finding cysts or cystlike bodies in cultures of T. conorhini and T. cruzi.

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TABLE 1. Mice of the genus Peromyscus: trap record, occurrence of infection and success in culturing

Mouse	Species	Sex	Weight (g)	Date captured	Trypanosomes (+ or 0)	Culture (+ or 0)
1	P. boylii	M	22.4	30 Sept. 1967	0	0
2	P. boylii	F	14.8	30 Sept. 1967	0	0
3	P. leucopus	F	26.4	30 Sept. 1967	0	0
4	P. boylii	F	13.3	30 Sept. 1967	0	0
5	P. truei	M	21.6	30 Sept. 1967	+	0
6	P. boylii	M	13.4	30 Sept. 1967	0	0
7	P. boylii	F	24.3	29 Oct. 1967	0	0
8	P. truei	F	15.7	29 Oct. 1967	0	0
9	P. boylii	F	20.6	19 Nov. 1967	0	Not inoculated
10	P. boylii	M	26.8	19 Nov. 1967	0	Not inoculated
11	P. truei	M	18.8	19 Nov. 1967	+	0
12	P. truei	M	20.2	19 Nov. 1967	0	Not inoculated

TABLE 1. Continued

Mouse	Species	Sex	Weight (g)	Date captured	Trypanosomes (+ or 0)	Culture (+ or 0)
13	P. boylii	M	17.0	19 Nov. 1967	0	Not inoculated
14	P. boylii	M	21.7	3 Dec. 1967	+	+
15	P. boylii	M	19.3	27 Dec. 1967	+	+
16	P. truei	F	21.4	27 Dec. 1967	+	+
17	P. boylii	M	21.4	27 Dec. 1967	0	Not inoculated
18	P. boylii	M	27.4	27 Dec. 1967	0	Not inoculated
19	P. truei	M	17.4	7 Jan. 1968	0	Not inoculated
20	P. truei	M	18.4	7 Jan. 1968	+	+

TABLE 2. Mouse 14 (P. boylii): all tubes inoculated 3 Dec. 1967, incubation at 22 C

Tube	Type of medium	Date of first examination	Forms	Days survived
14-1	NNN	4 Dec. 1967	trypanosomes, crithidias	5
14-2	NNN	4 Dec. 1967	trypanosomes, crithidias, rosettes	22
14-3	NNN	13 Dec. 1967	trypanosomes, crithidias	11
14-4	NNN	15 Dec. 1967	trypanosomes, crithidias	15
14-5	NNN	15 Dec. 1967	trypanosomes, crithidias	19
14-6	NNN	15 Dec. 1967	trypanosomes, crithidias	17
14-7	NNN	13 Dec. 1967	trypanosomes, crithidias	11
14-8	NNN	13 Dec. 1967	none	0
14-a-1	NNN (Mathis)	4 Dec. 1967	trypanosomes	6
14-a-2	NNN (Mathis)	9 Dec. 1967	trypanosomes, crithidias	10
14-a-3	NNN (Mathis)	15 Dec. 1967	none	0
14-2-4	NNN (Mathis)	15 Dec. 1967	none	0

TABLE 2. Continued

Tube	Type of medium	Date of first examination	Forms	Days survived
14-a-5	NNN (Mathis)	15 Dec. 1967	none	0
14-a-6	NNN (Mathis)	15 Dec. 1967	none	0
14-a-7	NNN (Mathis)	15 Dec. 1967	trypanosomes, crithidias	13

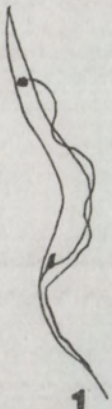
TABLE 3. Mouse 15 (P. boylii) and mouse 16 (P. truei): trypanosomes cultured on NNN medium

Culture	Date inoculated	Temperature of incubation (C)	Forms	Days survived
15-1	27 Dec. 1967	22	trypanosomes	2
15-1a, b	28 Dec. 1967	1.5	trypanosomes, crithidias	40
15-2	27 Dec. 1967	22	trypanosomes, crithidias	15
16-1	27 Dec. 1967	37	none	0
16-2	27 Dec. 1967	37	none	0
16-3	27 Dec. 1967	22	flagellated forms, crithidias	14
16-4	27 Dec. 1967	37	short forms, dividing types	6
16-5	27 Dec. 1967	22	trypanosomes, crithidias, cysts, rosettes, leptomonads, leishmanias	110
16-5-1	3 March 1968	22	rosettes, crithidias	20
16-6	27 Dec. 1967	22	contaminated, no growth	0

TABLE 4. Mouse 20 (P. truei): trypanosomes cultured on NNN and TSB media, inoculated 7 Jan. 1968

Culture	Medium	Temperature of incubation (C)	Forms	Days survived
20-1	NNN (sheep)	37	none	0
20-2	NNN (rabbit)	37	none	0
20-3	NNN (sheep)	22	trypanosomes, crithidias (inside clear structures)	2
20-4	NNN (sheep)	22	none	0
20-5	NNN (rabbit)	1.5	trypanosomes, crithidias	30
20-6	TSB	1.5	trypanosomes, crithidias (placed at 22 C on Jan 29, 1968), rosettes, leptomonads, leishmanias, cysts (successful subcultures)	85
20-7	TSB	22	trypanosomes, crithidias, rosettes, leptomonads, leishmanias, cysts, metacyclic forms	82

FIGURES 1-8. Sketches of Trypanosoma peromysci. 1. Trypanosomal stage from the blood of a mouse. 2. Dividing forms from culture 14-2. 3. Rosette form from culture 14-2 after incubation at 22 C for 14 days. 4. Dividing form from culture 16-4 incubated at 37 C. 5. Part of a rosette showing flagellated and nonflagellated individuals. 6. Cystlike body from culture 20-7. 7. Form with a partially complete undulating membrane from culture 20-6 after 94 days. 8. Ruptured cyst and whole cyst from culture 20-6-3.



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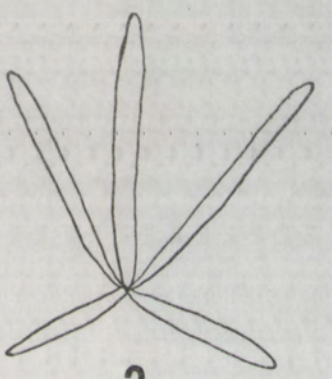

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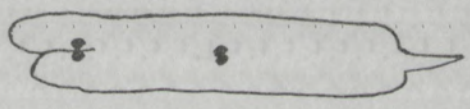

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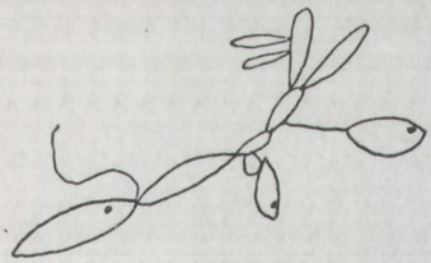

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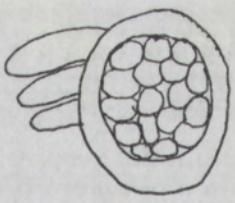

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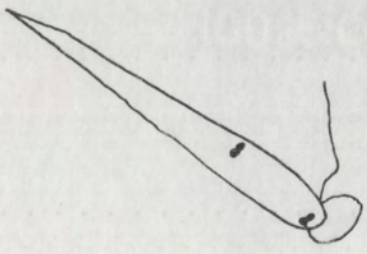

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FIGURES 9, 10. Photomicrographs of Trypanosoma peromysci.

9. Photomicrograph of a blood form (slide stained with Wright's-Giemsa stain). 10. Photomicrograph of a cystlike body.

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