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SERIAL PASSAGE OF *TRYPANOSOMA LEWISI* IN THE HETEROLOGOUS MOUSE HOST

II. DEVELOPMENTAL HISTORY DURING TRANSFER IN ADEQUATELY-FED HOSTS (*)

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Introduction

The blood trypanosome, *Trypanosoma lewisi,* is a highly host-specific parasite, and grows well only in rats but occasionally grows poorly in other animal hosts (Lincicome, 1958*a*).

It has been induced to grow in heterologous mouse hosts (Lincicome, 1955, 1957, 1958*a* and *b*) by supplementing the mouse with a daily quantum of serum from a normal rat. The significance of this observation has been reviewed by Lincicome (1959*a*).

Continuous consecutive passage of this trypanosome in the heterologous mouse host over a period of 3,5 years in calorically restricted mouse hosts supplemented with rat serum has been accomplished (Lincicome, 1959*a*). After 75 serial passages in these

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mice it was found possible to maintain the trypanosomes in mice that were fed a commercial laboratory diet *ad libitum*, plus a daily quantum of homologous serum.

The developmental history of *Trypanosoma lewisi* serially maintained in these normally fed mice forms the basis for this communication. A subsequent report will deal with assays of the organism during its growth in calorically restricted and adequately fed hosts to determine whether it has undergone significant metabolic adjustments while in heterologous hosts (Lincicome, 1959*b*).

Materials and methods

The parasite (*Trypanosoma lewisi*) employed here is designated the « C » isolate and originated during an experiment to test the ability of the « A » isolate of this species to grow in non-starved and normally fed mice after it has been serially transferred in calorically restricted mice for 75 passages (Lincicome, 1959*a*).

Since April 1956 when it first developed in normal mice it has been maintained continuously for a total of 220 consecutive transfers principally in albino and C3H mice by syringe passage of tail blood suspended in physiologic saline. It was voluntarily discontinued on 30 August 1958. Details of the transfer methods employed and estimations of infection levels may be found in a previous paper (Lincicome, 1959*a*).

Most mouse hosts were either stock albino (1) or C3H (2) strains of both sexes weighing 18-22 grams. A few beige mice were also used. These were of a highly inbred strain that had been developed and maintained for several years in this laboratory. All hosts were housed individually and given free access to water and a commercial laboratory biscuit (3).

One ml of normal rat serum was given intraperitoneally to each mouse daily from the day of inoculation of trypanosomes until the development of maximal parasitemia or until the next subsequent transfer was made. All sera were collected from apparently normal healthy rats, usually large males, of two strains (4). Sufficient quantities of sera to supply requirements for a week or 10 days were collected and stored at deep-freeze temperatures until used.

(1) Stock albino mice from the colony maintained at the Naval Medical Research Institute.

(2) Stock C_3H mice supplied from the animal laboratories of the National Institutes of Health, Bethesda, Md.

(3) Laboratory Chow, Purina Ralston Company, St-Louis, Mo.

(4) Sprague-Dawley and Long-Evans strains maintained in stock colonies at the Naval Medical Research Institute.

Results and comparisons

The numbers and distributions of host animals employed are summarized in Table I. Albino mice were used for passages 1 through 161 and again for passages 167, 168, 186, and 187. Beige mice were used for transfers numbered 162 through 166. The C3H mouse was utilized in all others.

TABLE I

Numbers of Normally Fed Mice Employed as Hosts for « C » Isolate of Trypanosoma lewisi

Passage	No. Animals Used	Passage	No. Animals Used	Passage	No. Animals Used	Passage	No. Animals Used
1-10	32	61-70	30	121-130	30	181-190	59
11-20	30	71-80	30	131-140	29	191-200	60
21-30	30	81-90	30	141-150	30	201-210	60
31-40	30	91-100	29	151-160	33	211-220	49
41-50	30	101-110	30	161-170	60		
51-60	30	111-120	30	171-180	60	Total	831

Observations of the developmental history of the « C » isolate were made on the :

1. duration of the parasitemic period in circulating tail blood ;
2. intensity of the parasitemia in circulating tail blood ;
3. interval required for the development of the observed maximal parasitemia ;
4. duration of the observed maximal parasitemia ;
5. interval to the next subsequent passage in mice ;
6. proportion of host animals that died ;
7. day of death of each host animal.

The essential data relevant to these observations are arranged in tabular form as averages per group of 10 consecutive transfers

beginning with the first in albino mice on 16 April 1956 through the 220th that terminated the study.

1. Duration of the Parasitemic Period

The data collected in Table II indicate that the length of the parasitemic period in normally fed mice was relatively stable throughout 220 serial transfers.

TABLE II

Duration of the Parasitemic Period in Circulating Tail Blood of Mice Data Expressed as Averages. Range of Observations in Parentheses

Passage	Days	Passage	Days	Passage	Days	Passage	Days
1-10	5.2 (5-8)	71-80	5.7 (4-8)	141-150	6.5 (5-9)	211-220	5-5 (3-8
11-20	5 2 (2-6)	81-90	5.9 (4-9)	151-160	6.5 (3-14)		
21-30	5.5 (4-9)	91-100	6.1 (4-10)	161-170	5 9 (4-8)	Totals	
31-40	5 4 (5-6)	101-110	5.9 (4-9)	171-180	6 0 (3-11)	1-100	5.6
41-50	5.4 (5-6)	111-120	6.4 (5-9)	181-190	5 8 (4-9)	1-200	5.9
51-60	6.0 (5-11)	121-130	5.9 (5-8)	191-200	5.2 (3-8)	1-220	5.8
61-70	5 8 (5-8)	131-140	6.2 (4-9)	201-210	5 4 (3-8)		

The variation in average length per each group of 10 transfers (5,2-6,5) overlapped that observed for the « A » isolate (6,2-6,8) and the « B » isolate (6,3-8,7) previously reported (Lincicome, 1959*a*).

The average parasitemic period for all 220 transfers was well below that of the « A » (5,8 vs. 6,5) and the « B » organism (vs. 7,9).

2. Intensity of the Parasitemia

Over one-half of the more than 800 animals used in this study developed maximum parasitemias of 4+ (Table III). This contrasts with the 77 % of animals which developed similar levels of infections with the « A » organism, but compares favorably with the 60 % observed for the « B » isolate (Lincicome, 1959*a*).

There is clearly an initial period of adjustment to the new heterologous environment extending over a time period of twenty passages (approximately 120 days) after which there appears to be perceptible improvement for the next 80 passages (about 460 days). The data in Table III also indicate that beyond the first hundred transfers there is relative stability in the numbers of animals that develop 4+ infections. This would suggest that adjustment of the parasite to its environment had reached a plateau.

Progressive adjustment of the « A » organism assumed a different pattern than the apparently gradual one observed here. When the « A » parasite was introduced into heterologous hosts 40 % of the albino mice developed 4+ infections within the first 10 passages, and 65 % had similar levels by the completion of 20 transfers (Lincicome, 1959*a*). Comparable development of the « C » line did not occur until after 50 passages had been completed. Timewise this relationship is roughly 130 days for the « A » isolate and something less than 300 days for the « C » parasite.

Further data relative to the percentages of animals developing less intense infections are presented also in Table III. They generally indicate that about a third of the hosts developed 2+ and another third had 3+ infections initially. By the end of the first hundred this relationship had not changed. An expected reduction in this pattern took place during the remaining 120 transfers and one-quarter of the animals had 3+ infections and only 15 % had 2+ infections. The shift from a 1/3 to a 1/6 relationship is accounted for by the slow adjustment indicated above in which a larger proportion of the animals had maximal infections.

3. Interval Required for the Development of the Observed Maximal Parasitemia

The data on the interval required for the development of the observed maximal (3+ and 4+) parasitemias were expressed as averages (in days) per unit of 10 consecutive passages. The range of this characteristic was 3,4-4,3 days with an overall average of 3,7 for 220 transfers.

At the completion of the first 100 passages the average interval was 3,6 days, and the similar average of the data from transfers 101 through 200 was 3,9.

These figures indicate relatively little variation in the time required for the development of maximal infections. This might be interpreted as indicative of a uniformity or stability of environ-

ment, or, adjustment to the environment, and/or uniformity in the initial population inoculated.

The data on this characteristic do not indicate any progression in adaptation to environment as was suggested for the « A » isolate (Lincicome, 1959*a*). The uppermost range of 10 passages unit averages of the « C » isolate was lower than the 4,4 days observed for the « B » organism.

4. Duration of the Observed Maximal Parasitemia

The duration of the observed maximal parasitemia in circulating tail blood of the mouse presents another characteristic for judging the heterologous environment provided by the mouse and/or the trypanosome population response to this environment.

Expressed as averages per unit of 10 consecutive passages the range of observations was 1,3 to 2,2 days. The average duration for the first 100 passages was 1,7 days, and for the second hundred, 1,8, with an overall figure for the 220 passages of 1,7.

These data contrast with those for the « A » and « B » isolates. For the former, during the first hundred transfers the length of maximum parasitemia was 2,4 days ; for the second, 2,2 and for the third, 2,1 suggesting an adaptation to the environment since the population increments also were progressive 65, 79, 85 % developing 4+ infections (Lincicome, 1959*a*). The average for all 300 passages for the « A » organism was 2,2 reflecting the greater length of the patency inherent in this relationship. The range of 1,7-2,9 days observed for the « A » parasite overlaped that for the « C » line.

The average length of the maximal patency period in the « B » isolate was well above that observed for either of the other two. The range of these observations likewise (2,1-4,3) was broader although falling within the limits of both the « C » and « A » organisms.

5. Interval to Next Subsecuent Passage in Mice

Transfer from one host to the next was made at a point during the development of the parasitemia when the parasite population was judged to be maximal. The intervals between introduction of the parasites and the next subsequent passage of the trypanosomes was judged as a possible measure of the character of the pattern of growth of *Trypanosoma lewisi* in the heterologous mouse host.

The average interval for the « C » isolate for the first 100 passages was 4 days (range : 3,7-4,2) ; for the second 100 passages, 3,9 (range : 3,5-4,3). The overall average for 220 passages was 3,9 with a range of 3,3 to 4,3.

This compares favorably with observations of the « A » isolate reported earlier, and warrants the conclusion that there was no difference between the two organisms in this respect.

The average of 4,9 and range of 4,6 to 5,4 days for the « B » isolate fall beyond the limits of either the « A » or « C » organism, and indicate further dissimilarities between the « B » parasite on the one hand and the « A » and « C » on the other.

6. Proportion of Host Animals that Died

The data for the percentage of host animals dying are arranged in Fig. 1.

The first 40 passages clearly show that the trypanosome has not reached population levels sufficient enough to produce death of the sheltering host. This is corroborated by the data on intensity of the parasitemia during this period (see Table III). The next 20 passages show a slowly rising death rate concomitant with a similar rise in intensity of the parasitemic response (Table III).

Data in Table III have suggested (see above) that there is relative stability in numbers of animals that develop 4+ infections beyond the first hundred transfers thus intimating that adjustment of the parasite has reached a plateau. Though this be an interpretation based upon the intensity of the parasitemia and thus may be considered a measure of the fitness of a population to its environment, it is not correlated with the observations here of the proportion of animals that died. After the period of about 20 transfers noted above there appears to be a cyclical fluctuation in which initially there is a rapid rise in the percentage of animals that die followed by a decline for twenty passages and then another rapid rise (Fig. 1). This pattern is repeated with varying intervals until about the 161/170 passages period when C3H mice were first introduced into the protocol. The rising and falling pattern thus established is broken and the curve plainly shows a relatively sustained rise. This is interpreted to mean that the environment provided by the heterologous C3H mouse is qualitatively different from that of the albino mouse. This is not reflected in other observations reported here on the duration of the parasitemic

TABLE III — *Intensity of the Parasitemic Period. Data Expressed as Percentages of Animals that Developed Each Level of Infection within Groups of 10 Passages*

Parasitemia Level	Passage											
	1/10	11/20	21/30	31/40	41/50	51/60	61/70	71/80	81/90	91/100	Total 1/100	101/110
4 +	0	3	27	37	20	43	33	73	63	45	34	83
3 +	31	37	27	47	53	27	33	17	21	31	33	10
2 +	38	47	46	10	23	27	27	10	14	21	26	7
1 +	22	13	0	6	4	3	7	0	0	3	6	0
0	9	0	0	0	0	0	0	0	0	0	1	0
	111/120	121/130	131/140	141/150	151/160	161/170	171/180	181/190	191/200	201/210	211 220	Total 1/220
4 +	77	90	66	63	45	72	80	54	77	85	90	59
3 +	13	10	24	17	18	27	13	20	18	10	5	23
2 +	3	0	7	13	31	1	7	19	5	5	5	15
1 +	7	0	3	7	6	0	0	7	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	< 1

period, intensity of the parasitemia, the interval required to reach maximal patency, or the interval to the next subsequent mouse passage. The dash lines in Fig. 1 show the considerable difference in average percentages of animals dying between the first and second hundred passages.

The « A », « B », and « C » isolates have shown similar behavior as judged by the proportion of animals that died during the study. The 62 % observed for the « A » line (Lincicome, 1959*a*) was inter-

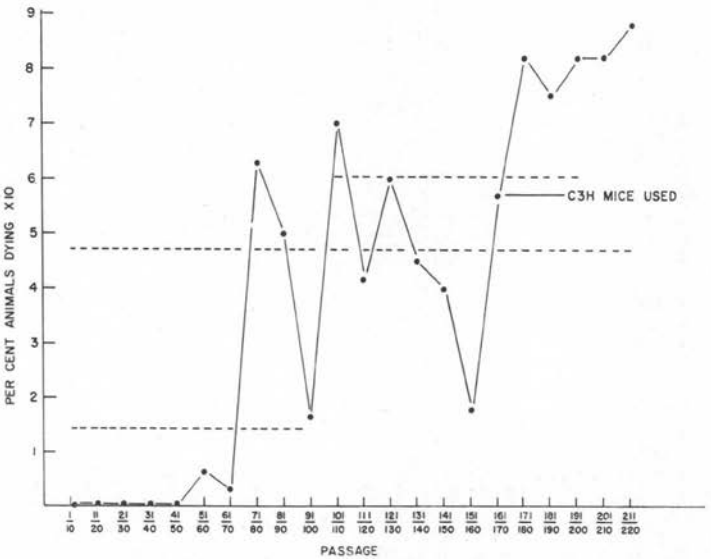

FIG. 1. — Percentage of host animals of *Trypanosoma lewisi* (« C » Isolate) dying in relation to the number of serial mouse passages. Dash lines represent averages per indicated intervals.

mediate between the 47 % of the « C » and the 68 % of the « B » organism. With both the « A » and « C » strains there did not appear to be a correlation between the numbers of host animals that died and other characteristics used to judge the performance of this trypanosome.

7. Day of Death of Each Host Animal

There were no deaths of host animals for the first 50 passages (Fig. 2). Subsequent to this the curve constructed for the average day of death per unit of 10 passages followed a relatively narrow

pattern of rising and falling. This pattern is not correlated with any significant protocol variation except that the initial observations indicate further evidence of an adjustment period.

This sequence stands in contrast with previous findings with the « A » and « B » isolates (Lincicome, 1959*a*).

No great change in time of death was observed between the first and second hundred transfers (see dash lines on Fig. 2). The averages for the first and second hundred passages and the

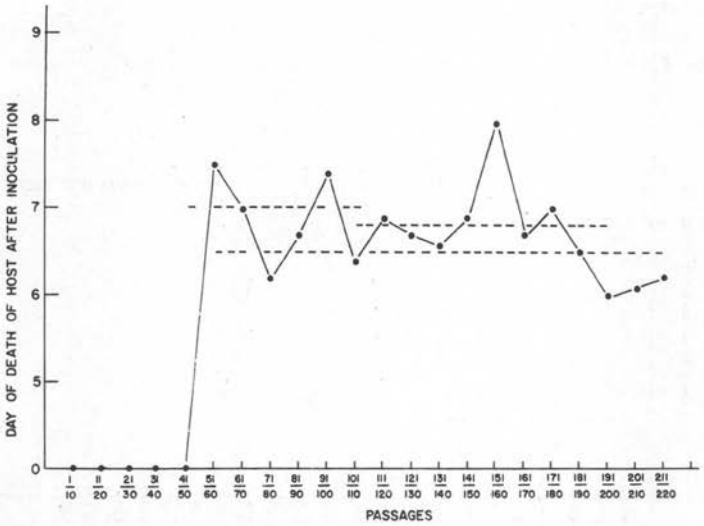

FIG. 2. — Day of death of hosts of *Trypanosoma lewisi* (« C » Isolate) in relation to the number of serial mouse passages. Dash lines represent averages per indicated intervals.

combined observations over all 220 passages are within a narrow limit thus supporting the view that little or no evolution of this characteristic occurred throughout the whole of the study.

The data for the « A » isolate suggest the antithesis of that observed for the « C » organism in that the pattern of average death per units of 100 passages declined : 7,6, 7,3, 6,7, suggesting and correlating with an increase in population increments.

The average day of death of host animals for the « B » isolate was 8,1 and thus far exceeds the observations on either of the other two organisms.

The evidences from 1) the proportion of host animals that died, 2) the intensity of the parasitemia, and 3) the day of death of host

animals support the general interpretation that the « C » strain of *Trypanosoma lewisi* has undergone a gradual adjustment to live within the heterologous mouse environment. Observations of the 1) interval required for development of the observed maximal parasitemia, 2) the duration of the observed maximal parasitemia, and 3) the interval to the next subsequent passage in mice indicate, however, that there is little evolutionary change in the relationship between parasite and its sheltering host throughout the whole 220 consecutive passage generations.

The nature of the gradual adjustment noted above is, of course, not elucidated by these studies, since they were designed only to determine whether this strain (« C ») of *Trypanosoma lewisi* could be consecutively maintained in unstarved hosts, and whether during the course of transfer there was any measurable change in developmental history.

It is reasonable to expect that were this trypanosome to undergo a significant change in its metabolic requirements, it should be able to maintain itself in an heterologous host after a period of adjustment without the aid of homologous serum supplements. Theoretically this change should be manifested also in an alteration of the normal pattern of development when the parasite is returned to its native host.

If *Trypanosoma lewisi* does not evolve metabolically during the experimental association with an heterologous host thereby maintaining its requirement for homologous serum supplements, the possibility of determining what fraction of the normal host serum is requisite for its maintenance under heterologous conditions appears encouraging.

From time to time during this study the trypanosomes have been assayed to determine 1) whether they would flourish in mouse hosts without the rat serum supplement, and 2) whether they retained their native ability to develop in the rat host. The results of these studies will be reported in a subsequent paper in this series (Lincicome, 1959*b*).

Summary

1. An analysis is presented of the developmental history of *Trypanosoma lewisi* through 220 serial transfers in normal mice supplemented with rat serum, after having been consecutively transferred through 75 passages in calorically restricted mice.

2. The length of the parasitemic period in mice was stable. The average per group of 10 transfer units varied from 5,2 to 6,5 days.

3. Approximately 60 % of the 831 animals used developed maximal 4+ infections. The first hundred passages show evidences of major adjustments of the trypanosome to an heterologous environment. The second hundred passages show relative stability of the numbers of host animals that succumb.

4. An average of 3,7 days was required for the interval from inoculation to the observed maximal parasitemia.

5. There was little variation in the duration of the observed maximal parasitemia (Av. 1,7).

6. An average of 3,9 days elapsed to the next subsequent passage in mice.

7. The data for the characteristic of the proportion of host animals that died corroborate the observation that the trypanosome has made an adjustment to its new environment over the period of the first 100 consecutive transfers. The last hundred odd transfers show a cyclically rising and falling pattern, suggesting more or less stability of conditions.

8. Little or no evolution was observed in the day of death of host animals during the course of the study.

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