CONCERNING CHANGES IN THE BIOLOGICAL PROPERTIES OF TRYPANOSOMA LEWISI PRODUCED BY EXPERIMENTAL MEANS, WITH ESPECIAL REFERENCE TO VIRULENCE.*

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During the past few years a great deal of interest has been manifested in biological variations, or mutations, in trypanosomes induced by experimental procedures. That even such simple manipulations as are practiced in maintaining a stock strain of trypanosomes might lead to an appreciable degree of biological alteration in some trypanosomes was rather strongly suggested to me by an instance of exalted virulence in a strain of *Trypanosoma lewisi,* an account of which was published about a year ago.[1]

While others have observed similar examples of virulence in *Trypanosoma lewisi,* notably Jürgens,[2] no satisfactory explanation has been offered for such occurrences. By experimental procedure, however, very marked alterations in the biological properties of this organism have been produced. As early as 1909 Wendelstadt and Fellmer[3] succeeded in producing morphological alterations and an increase in the virulence of *Trypanosoma lewisi* by a series of passages through cold-blooded animals. More recently, the experiments with a reinforced virus, initiated by Roudsky,[4] have demonstrated clearly the possibility of developing a strain of *Trypanosoma lewisi* of a highly virulent character, capable even of serial infection in mice.

Especial importance may be attached to the use of *Trypanosoma lewisi* in such studies on variation, as the relations obtaining between

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[1] Brown, W. H., *Jour. Exper. Med.,* 1914, xix, 406.

[2] Jürgens, *Arch. f. Hyg.,* 1902, xlii, 265.

[3] Wendelstadt, H., and Fellmer, T., *Ztschr. f. Immunitätsforsch., Orig.,* 1909, iii, 422; 1910, v, 337.

[4] Roudsky, D., *Compt. rend. Soc. de biol.,* 1910, lxviii, 421, 458.

host and parasite in the instance of this organism are different from those existing with most laboratory strains of trypanosomes, in that *Trypanosoma lewisi* is one of a few constantly carried in its natural host. While seeking an explanation of our specific problem of virulence, therefore, the attempt was made to approach the problem from a fundamental biological viewpoint with the belief that such information as could be obtained might prove of broader application.

SOURCES AND NATURE OF MATERIALS.

In the particular instance of variation that occurred in our stock strain of *Trypanosoma lewisi,* the indications were that either the mode of passage, the character of the rats, or both factors had been instrumental in inducing the change. Accordingly, a simple series of experiments was begun to test the influence of the rate of passage, passage during different periods of the infection, the dose of trypanosomes used, and the character of the rats, as measured by weight, upon succeeding generations of trypanosomes. Two strains of *Trypanosoma lewisi* were used in the tests. One of these, designated as strain I, was the so called pathogenic strain, and the other, strain V, had just been obtained from a natural infection in a young rat. This rat was one of seven infected rats out of a lot of thirty-six obtained from a dealer. The infections in these rats had progressed without symptoms, indicating that the organism was of a relatively harmless type. Very distinct differences between these two strains of *Trypanosoma lewisi* persisted through four parallel series of experiments on white rats. The first series of rats, tables I and II, comprised the stock transfers. These rats were mostly large. They were all inoculated intraperitoneally with two drops of tail blood in 0.5 of a cubic centimeter of salt solution. The rate of passage was necessarily irregular but relatively slow, and in some instances there were several groups of rats inoculated from a common source infection at different periods of the infection, as in generations VI, VII, VIII, and X (table I).

In the second series (tables III and IV) large rats were also used. The inoculations were carried out as in the first series. The transfers were made, however, as near the height of multiplication as pos-

sible, except in the last five generations of strain V; the rate of passage was relatively rapid and uniform.

The third series of experiments, given in tables V and VI, differed from the second only in the use of small rats. The rate of passage in the last seven generations of strain V was intentionally varied here as in series 2.

In the fourth group small rats were used as far as possible (tables VII and VIII), and were inoculated in pairs. At the height of multiplication one animal was killed and its defibrinated blood, up to 0.5 of a cubic centimeter, was injected intraperitoneally into each of the next two rats. Thus the rats in the records do not represent a series of direct transfers but are, as it were, only the controls of the series.

In all these animals accurate records were kept of the course of infections. The blood was examined daily and weights were recorded at least once a week for thirty days. Observations were then discontinued in all except the stock series (series 1) where observations were continued until the termination of the infection.

CHANGES PRODUCED IN THE INFECTION CYCLE.

Incubation Period.—Other conditions being kept relatively constant, the rate at which *Trypanosoma lewisi* was passed from rat to rat was found to exercise a distinct influence upon the various phases of the infection cycle in succeeding generations of the organism. The earliest evidence of such an effect was seen in the incubation period. An examination of table I shows that of thirty-five rats in only three was the incubation period less than two days, and these rats had been inoculated from infections of seven and ten days' duration. There were six other rats in the series inoculated at relatively the same periods that showed longer periods of incubation. Again, with strain V, table II, we find three rats with incubation periods of one day. Here, however, two of these followed inoculation from an infection of twenty-nine days' duration. Again, there were three rats inoculated from infections of only ten days' duration, or less, with incubation periods of two days. That the tendency of rapid passage was undoubtedly to shorten the incubation period in suc-

TABLE I.

Slow Passage of Small Doses of Virus in Large Rats.

Trypanosoma lewisi, Strain I, Series 1.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	85	5th	5	++	Marked	11	Death	
II	2	175	10th	4	++	Marked	11	Death	
	* 3	132	10th	4	++	Marked	15	Death	
III	4	116	12th	5	+++	Marked	8	Death	
	* 5	150	12th	4	++	Marked	12	Death	
IV	* 6	146	8th	3	+++	Marked	—	Killed	Used to test virulence of strain. Killed after 12 dys.
	7	176	8th	—	—	—	—	—	
	8	165	8th	—	—	—	—	—	Rats 7 and 8 immune.
V	9	70	10th	2	+++	Marked	37	Killed	For bacteriological and pathological study.
	*10	72	10th	2	++	Moderate	107	Recovery	Late symptoms marked.
VI	11	79	13th	2	++	Moderate	—	Killed	To test virulence of strain.
	12	162	13th	4	+	Slight	22	Recovery	Experiments actually began with this generation.
	13	85	41st	5	+++	Marked	18	Death	
	14	79	61st	5	++	Moderate	14	Recovery	
	*15	127	61st	6	++	Slight	58	Recovery	
VII	*16	135	29th	5	+++	Marked	12	Death	
	17	93	46th	—	—	—	—	—	Immune.
	*18	127	46th	5	++++	Marked	49	Recovery	Condition fair.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
VIII	19	114	10th	1	+++	Marked	13	Recovery	Rats 19 and 20 inoculated from 16; others from 18. Rat 19, condition poor.
	20	63	10th	1	+++	Marked	23	Death	
	21	141	12th	2	++	Moderate	44	Recovery	Condition good.
	22	141	12th	2	++	Moderate	76	Recovery	Condition good.
	23	143	21st	3	++	Moderate	34	Recovery	Condition fair.
	24	92	21st	3	+++	Marked	22	Death	
	*25	145	32d	3	+++	Marked	35	Death	
IX	*26	150	7th	1	+++	Slight	61	Recovery	Condition good.
	27	140	18th	—	—	—	—	—	Immune.
X	28	146	28th	2	++	Slight	10	Recovery	Condition good.
	29	152	28th	2	+	Slight	10	Recovery	Condition good.
	*30	130	37th	6	+++	Marked	37	Death	
	*31	126	37th	5	+++	Moderate	130	Death	Late symptoms marked.
	32	110	41st	—	—	—	—	—	Immune.
	33	118	41st	5	+++	Moderate	32	Death	Late symptoms marked.
XI	34	123	27th	4	+++	Marked	21	Death	Rat 34 inoculated from 30.
	35	119	43d	5	+++	Marked	108	Death	Rat 35 inoculated from 31.

In all the tables where several rats are shown in one generation of transfers, the rats in the direct series are marked with an asterisk (*).

The figures in the column marked "day of passage" indicate the time elapsing between successive inoculations and refer particularly to the duration of infection in the rat from which a given rat was inoculated. All times estimated are from the time of inoculation.

Relative numbers of trypanosomes in the blood are indicated thus: Very few, +; few, ++; many, +++; great many, ++++.

cessive generations of trypanosomes may be seen by comparing this phase of the infection cycle of the rats in tables I and II with those in the tables following. Still, it must be recognized that the incubation period in any specific instance may be influenced to a great extent by such factors as the age of the infecting organism and the number of the organisms injected.

In this connection a distinction must be drawn between true and false incubation,—a fact that seems to have escaped attention in the literature. Where large numbers of trypanosomes are used to infect an animal, it is not uncommon to find organisms in the blood within a few hours. In the case of the adult forms of *Trypanosoma lewisi* it is easy to show that such an invasion of the blood is not a true incubation, as the type of organism is the same as that injected and the numbers of the organisms subsequently diminish or may entirely disappear from the blood for a day or more before the true invasion of young forms occurs. Where young or multiplying trypanosomes are used, the distinction between true and false incubation becomes arbitrary or fails.

Periods of Multiplication.—As with the period of incubation, rapid passage of *Trypanosoma lewisi* tends to advance the time at which multiplication begins in the blood of the rat. In the tabulated records of the rats in series 2, 3, and 4, the figure in the column indicating the "day of passage," in most instances, also indicates the day on which the height of multiplication was reached in the preceding rat. In many of these rats, multiplication was well advanced by the end of the second day after injection and completed by the end of the fourth day. This is in sharp contrast to the usual phase of multiplication, both as to time and duration of the cycle, as well as to the period elapsing between incubation and multiplication. As in the case of incubation, only a few passages were necessary to develop the tendency to shorten the gap between incubation and multiplication, to accentuate the rate of multiplication, and to favor an early termination of the cycle.

Duration of Infection.—Infections of *Trypanosoma lewisi* tend to be chronic, to last for thirty days or more, but exceptions to this rule are not at all uncommon; the usual termination is in recovery. In series 1 of strain V, after the first generation, only one rat out of

TABLE II.

Slow Passage of Small Doses of Virus in Large Rats.

Trypanosoma lewisi, Strain V, Series 1.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	* 1	117	?	4	+++	Slight	20	Recovery	Condition good.
	2	92	?	5	+++	Slight	22	Recovery	Condition good.
	3	83	?	5	+++	Slight	—	Killed	Killed 8th dy. to test virulence of strain.
II	4	90	4th	2	++++	Slight	86	Recovery	
III	5	90	23d	—	—	—	—	—	Immune.
	* 6	110	39th	3	++++	Moderate	47	Recovery	Condition good.
IV	7	93	34th	4	++++	Moderate	48	Recovery	Condition good.
V	8	153	29th	1	++	Moderate	59	Death	Rats 8 and 9 were infested with lice(?).
	9	145	29th	1	++	Moderate	80	Death	
	*10	167	38th	3	+++	Marked	34	Recovery	Condition poor.
VI	*11	147	15th	2	++++	Marked	14	Death	
	12	165	18th	—	—	—	—	—	Immune.
VII	*13	130	10th	2	+++	Moderate	35	Recovery	Condition fair.
	14	142	10th	2	+++	Moderate	35	Recovery	Condition fair.
VIII	15	154	21st	2	++++	Moderate	27	Recovery	Condition very poor.
IX	16	123	18th	2	++++	Moderate	31	Death	
	17	114	18th	—	—	—	—	—	Immune.
X	18	136	10th	1	+++	Moderate	33	Recovery	Condition poor.
	19	127	29th	2	++++	Marked	45	Recovery	Condition poor.

TABLE III.

Rapid Passage of Small Doses of Virus in Large Rats.

Trypanosoma lewisi, Strain I, Series 2.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	111	7th	2	+	Moderate	10	Recovery	Virus from rat 11, series 1. Condition fair.
II	2	117	6th	4	+	Marked	10	Recovery	Condition poor.
III	3	135	7th	2	+++	Marked	30+	Survival	Condition good.
IV	4	211	4th	2	+++	Moderate	30+	Survival	Condition fair.
V	*5	94	4th	1	++	Slight	15	Recovery	Condition fair.
	6	95	4th	1	++	Slight	22	Recovery	Condition fair.
VI	7	102	5th	2	+	Slight	11	Recovery	Condition fair.
VII	8	200	4th	1	++	Slight	30+	Survival	Condition good.
VIII	9	122	3d	2	+++	Slight	30+	Survival	Condition good.
IX	10	150	4th	1	+	Marked	6	Recovery	Condition poor.
X	11	114	3d	3	+	Marked	13	Recovery	Condition poor.
XI	12	216	3d	5	++	Marked	12	Recovery	Inoculated from rat 13, series 3. Loss of weight, 46 gm. in 12 dys.

sixteen recovered within the thirty day period of observation (table II). In series 2, 3, and 4, however, where rapid passage was practiced, the last four rats, seven of the last nine, and five of the last six, respectively, recovered within the thirty day period.

In strain I the influence of passage upon the duration of the infection was not so clearly shown and there was only one series of rats, series 2, in which any consistent or definite course of alteration was manifest when compared with series 1. Other factors, which will be considered later, undoubtedly served to mask much of the effect of passage in this strain.

The results obtained with strain V, however, show very clearly what changes may be produced in the several phases of the infection cycle of an ordinary strain of *Trypanosoma lewisi* by the use of only the simplest experimental procedures. Mere regulation of the rate of passage of the virus from one rat to another seems sufficient so to alter the biological properties of *Trypanosoma lewisi* as to change an infection that is usually chronic into one that may be regarded as acute, or *vice versa*. These changes occurred, in my experiments, irrespective of the character of the rats used or of the dose of the infecting organisms.

CHANGES IN VIRULENCE.

The basis for estimating the virulence of *Trypanosoma lewisi* in these experiments was the incidence, especially serial incidence, and degree of such symptoms of intoxication as stupor, weakness, loss of weight, and anemia, together with the mortality definitely attributable to infection with *Trypanosoma lewisi;* very little account was taken of isolated instances of severe intoxication or even death.

In series 1 of strain I, the records show that infections with marked symptoms and even death occurred in all except one of the eleven generations of transfers. The virulence of the strain was at its height in the first three generations, though strongly evident again in the last two generations.

In the second series of large rats where the virus was passed rapidly, virulence was much less evident than in series 1. There were, however, well marked symptomatic disturbances in the first

three rats of the series. Even symptoms were then absent until the last two generations when they again became marked. The young rats of the third series showed a larger proportion of severe infections and a few deaths. Crossed infection at the termination of series 2 and 3 showed, however, that there was no appreciable difference in the character of the trypanosomes in the two series at that time (compare rats 11 and 12, series 2, and 13 and 14, series 3). The added influence of large doses of trypanosomes, as in series 4, produced no further alteration in the character of the infection except to insure a consistently shortened incubation period and a greater number of trypanosomes in the peripheral blood. No pronounced virulence was developed by strain V within the limits of these experiments, although the rats of series 1 and 3 showed a gradually increasing number of infections with distinct evidence of intoxication. This was especially evident in the last half of series 3, where the rate of passage was changed with a view to increasing the virulence of the organism. To a less degree, the other series showed a similar increase. The fluctuation in the character of the infections throughout was such as to suggest a cyclic series of changes,—a condition evident also with strain I.

A final factor that appeared to exercise a considerable influence upon the nature of the infection produced by *Trypanosoma lewisi* was the time at which the transfer of the trypanosomes was made. From table I it will be seen that in generation VI three sets of rats were inoculated. Two rats, inoculated from an infection of thirteen days' duration with active agglomeration in progress, developed mild infections. Another rat, inoculated from the same source on the forty-first day of infection, developed a severe infection which terminated fatally, while two other rats inoculated from the same rat on the sixty-first day of infection developed relatively mild infections. A similar condition was observed in generation VIII. Rats 19 and 20, inoculated from rat 16, both showed severe infections. One of these rats recovered after an acute infection and the other succumbed. Rats 21 and 22, inoculated from rat 18 on the twelfth day of infection, developed comparatively mild infections, while the infections in two of three other rats, inoculated from the

TABLE IV.

Rapid Passage of Small Doses of Virus in Large Rats.

Trypanosoma lewisi, Strain V, Series 2.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	94	4th	2	++++	Slight	30+	Survival	Virus from rat 3, series 1. Condition good.
II	2	92	4th	1	++++	Slight	30+	Survival	Condition good.
III	3	90	5th	2	++++	Slight	30+	Survival	Condition good.
IV	4	93	4th	1	++++	Moderate	30+	Survival	Condition fair.
V	5	140	3d	1	+++	Slight	30+	Survival	Condition good.
VI	6	115	4th	1	+++	Slight	30+	Survival	Condition good.
VII	7	174	3d	1	+++	Slight	30+	Survival	Condition good.
VIII	8	215	3d	2	+++	Moderate	15	Death	
IX	9	210	4th	1	+++	Marked	—	Killed	Killed after 7 dys. to test virulence of strain.
X	10	217	4th	1	+++	Moderate	28	Recovery	Condition fair.
XI	11	145	3d	1	++++	Slight	30+	Survival	Condition fair.
XII	12	137	4th	1	+++	Marked	18	Death	
XIII	13	132	5th	1	++	Moderate	30+	Survival	Condition fair.
XIV	14	140	6th	2	+++	Moderate	18	Recovery	Condition fair.
XV	15	122	8th	2	++++	Moderate	14	Recovery	Condition poor.
XVI	16	143	8th	2	+++	Moderate	14	Recovery	Condition fair.
XVII	17	110	11th	4	++	Moderate	28	Recovery	Condition poor.

same source on the twenty-first and thirty-second days of infection, terminated fatally. Again, in generation X similar results were obtained.

Similar experiments with strain V were inadequate to warrant independent interpretation, but, as far as they went, they were in harmony with those obtained with strain I.

CHANGES IN MORPHOLOGY.

This phase of the subject of biological variation of *Trypanosoma lewisi* has been discussed in detail in another paper.[5] It seems necessary, therefore, only to point out that the variations of morphology observed occurred mainly in infections of considerable severity, indicating a measure of interrelation between virulence and morphological variation.

Before entering upon a discussion of the experiments already described, mention should be made of still another group of experiments, the details of which must be omitted on account of some uncertainty as to their end results. This series of experiments comprised two groups of rats, large and small, with each of the two strains of *Trypanosoma lewisi.*

In these rats the infection was allowed to progress until recovery seemed imminent, when the rat was killed and 0.5 of a cubic centimeter of its defibrinated blood injected into the next rat. With the large rats of both strains and with the small rats of strain I, the trypanosomes were propagated for only a few generations when infection failed, presumably due to the use of immune rats. Two of the series had been interrupted in this manner before it occurred to us to test the immunity of these rats to the stock strain. When infection failed to develop in the third series, after two weeks, this rat was inoculated with the stock strain of *Trypanosoma lewisi* and promptly developed an infection. The possibility of an attenuation by the slow passage of large doses of trypanosomes was neither excluded nor proven, but the most plausible explanation of this result seems to be the transference of sufficient immune bodies with the trypanosomes to protect the new host against infection. This point raises a question of practical importance as to the propriety of a procedure so often employed in attempting to recover trypanosomes

[5] Brown, W. H., *Jour. Exper. Med.,* 1914, xix, 562.

TABLE V.

Rapid Passage of Small Doses of Virus in Small Rats.

Trypanosoma lewisi, Strain I, Series 3.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	93	7th	2	+	Moderate	10	Recovery	Virus from rat 11, series 1. Condition poor.
II	2	91	6th	5	+	Marked	10	Recovery	Condition poor.
III	3	60	7th	3	+++	Marked	30+	Survival	Condition poor.
IV	4	73	4th	2	+++	Marked	—	Killed	Killed after 5 dys. to start series 4.
V	5	70	4th	1	++++	Moderate	30+	Survival	Condition fair.
	*6	68	4th	1	++++	Moderate	30+	Survival	Condition fair.
VI	7	46	5th	1	+++	Marked	26	Death	
VII	8	60	3d	1	++	Moderate	30+	Survival	Condition fair.
	9	60	4th	1	++	Moderate	30+	Survival	Condition fair.
VIII	10	79	3d	2	+	Slight	30+	Survival	Condition good.
IX	11	46	4th	2	++	Marked	20	Death	
X	12	39	3d	1	+++	Slight	22	Recovery	Condition good.
XI	13	51	3d	1	++	Marked	7	Death	
XII	14	63	5th	2	+++	Marked	6	Death	Inoculated from rat 11, series 2.

from an animal suspected of being infected; namely, the transference of large amounts of blood containing but few trypanosomes and an unknown amount of immune bodies. Unfortunately, we have been unable to investigate this subject further.

DISCUSSION.

The experiments have necessarily covered a wide field of investigation in order to supply *indications* of the influence of a number of simple factors upon the biological properties of *Trypanosoma lewisi,* and must be so viewed. The conclusions that may be reached on some points are quite clear, while on others there is still some uncertainty.

It is perfectly obvious that, within certain limits, by regulating the time and rate at which the trypanosomes are passed from rat to rat, we may so alter the character of *Trypanosoma lewisi* as to change the course of the infection cycle completely. The development of a strain producing short or acute infections can be accomplished with greater certainty than the intentional reversion of such a strain to a consistently chronic type, or the maintenance of a chronic strain as such; the single factor of rapid passage seems sufficient to accomplish the transformation of a chronic into an acute strain, while in the development or maintenance of a chronic strain the essential conditions are more difficult of control. The occasional short infection in an exceptionally resistant rat, or the occasional severe infection in a susceptible rat, may compel an earlier transfer than would be desirable. Still more difficult to encompass are the cyclic changes in the character of the trypanosomes produced by immunological reactions in the rat's blood during the chronic phase of infection. In a broad sense, the biological status of the trypanosomes at any particular time during an infection may be regarded as only a resultant of these immunological reactions. Since the phenomenon of agglomeration constitutes our only guide to these reactions, the choice of a time when the biological status of the trypanosomes favors our purpose becomes a matter of extreme difficulty.

When we come to consider the question of virulence, this constantly changing status of *Trypanosoma lewisi* in the rat's blood

TABLE VI.

Rapid Passage of Small Doses of Virus in Small Rats.

Trypanosoma lewisi, Strain V, Series 3.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	55	4th	2	++	Slight	• ——	Killed	Virus from rat 3, series 1. To start series 4.
II	2	78	4th	1	+	Slight	30+	Survival	Condition good.
III	3	51	5th	2	+++	Marked	10	Death	
IV	4	62	4th	1	++++	Marked	30+	Survival	Condition poor.
V	5	62	3d	1	++++	Marked	30+	Survival	Condition fair.
VI	6	41	4th	1	++++	Moderate	30+	Survival	Condition fair.
VII	7	41	3d	1	++++	Marked	28	Death	
VIII	8	38	7th	1	++++	Marked	27	Death	
IX	9	43	4th	1	++++	Marked	30+	Survival	Condition very poor.
X	10	48	3d	1	++++	Moderate	25	Recovery	Condition good.
XI	11	39	4th	1	+++	Slight	24	Recovery	Condition good.
XII	12	49	5th	1	+++	Moderate	24	Recovery	Condition good.
XIII	13	42	6th	1	++++	Marked	18	Recovery	Condition fair.
XIV	14	38	8th	1	++++	Marked	20	Recovery	Condition fair.
XV	15	54	12th	1	+++	Moderate	21	Recovery	Condition fair.
XVI	16	74	8th	2	++++	Moderate	21	Recovery	Condition good.
XVII	17	63	3d	1	+++	Marked	26	Death	
XVIII	18	65	18th	2	++++	Marked	7	Death	

assumes even greater importance. Apparently the conditions to be met in maintaining or building up a virulent strain of *Trypanosoma lewisi* are indicated by the conditions obtaining in severe infections, of which I have observed three main types.

In one small group of such infections large numbers of trypanosomes persist in the blood with but scant evidence of their destruction. These infections are chronic and differ from the usual infection only in degree.

In the second and largest group the number of trypanosomes is never great, and degeneration and phagocytosis of trypanosomes are comparatively prominent. These infections may terminate very early,—usually in recovery,—or when multiplication persists beyond the accustomed limits the infection is more prolonged and not infrequently terminates fatally.

In the third and most important group multiplication is extremely active and irregular in character; the blood swarms with trypanosomes, and degeneration and disintegration of trypanosomes are marked; there is a marked leucocytosis, and the blood contains a large number of active phagocytes. Death of the rat usually occurs while multiplication is still active; recovery is exceptional.

If the last group be taken as the highest development of the virulent type, three factors in the infection become significant: vigorous reproduction, limited vitality of the trypanosomes, and a strong defensive (?) response on the part of the host. The association of vigorous reproduction with weakened resistance to destruction may appear somewhat paradoxical. There appears to be some distinction, however, between the mechanism limiting multiplication and that causing the destruction of trypanosomes in the rat's blood. In the usual infection of *Trypanosoma lewisi* we have evidence of what I must regard as two distinct classes of immunological reaction, one of which is concerned in checking the multiplication of trypanosomes, as ordinarily understood, and the other in their destruction. That these phenomena are in reality separable is shown by the fact that in some infections multiplication in the peripheral blood may be completely checked early in the infection with no appreciable decrease in the numbers of trypanosomes or other evidence of their destruction for weeks or even months. On the other hand, in certain infections, as indicated above, active multiplication may

TABLE VII.

Rapid Passage of Large Doses of Virus in Small Rats.

Trypanosoma lewisi, Strain I, Series 4.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	74	5th	1	+++	Slight	30+	Survival	Virus from rat 4, series 3.
II	2	102	4th	1	+++	Slight	30+	Survival	
III	3	57	2d	1	++++	Marked	30+	Survival	Condition very poor.
IV	4	200	2d	1	++++	Marked	17	Death	
V	5	62	3d	1	+++	Slight	30+	Survival	Condition good.
VI	6	58	2d	1	+++	Slight	30+	Survival	Condition good.
VII	7	67	2d	1	++++	Moderate	25	Recovery	Condition fair.
VIII	8	63	3d	1	+++	Slight	30+	Survival	Condition good.
IX	9	48	2d	1	+++	Slight	30+	Survival	Condition good.
X	10	70	3d	1	+++	Moderate	30+	Survival	Condition poor.
XI	11	61	3d	1	+++	Marked	6	Death	
XII	12	53	3d	1	+++	Slight	25	Recovery	Condition fair.
XIII	13	48	3d	1	+++	Slight	30+	Survival	Condition fair.
XIV	14	64	4th	1	+++	Moderate	30+	Survival	Condition fair.
	15	56	4th	1	+++	Moderate	30+	Survival	Condition fair.

continue beyond its usual limits, while the number of trypanosomes in the peripheral blood continually diminishes with abundant evidence of vigorous destruction.

The essential elements of a spontaneously acquired virulence of *Trypanosoma lewisi* appear, therefore, to be closely related to immunological reactions and to be dependent upon an appreciable degree of reproductive fastness, strong antigenic properties, and weakened resistance to destruction. Such an hypothetical basis for a naturally acquired virulence or for a virulence developed in *Trypanosoma lewisi* as the result of a system of passage obviously constitutes a state of unstable balance of the biological properties of the organism. It is not inconceivable, however, that such a condition might be maintained or developed by a fortunate system of passage that takes due account of the existing biological status, or balance, of the organism to be dealt with. For example, strain V of my experimental series was a normally balanced strain and showed a consistent response to the experimental procedures employed. Strain I, on the contrary, was highly unstable so that under the same experimental conditions as strain V its fluctuations were frequent and sharp with but little persistent tendency to change in any given direction.

Moreover, reference should be made to the animal equation in experimental modification of *Trypanosoma lewisi.* As far as resistance to infection is concerned, small rats seemed to offer less resistance or were more susceptible to intoxication than large rats, as others have repeatedly observed. Theoretically, it should be possible to utilize this factor, since in its final aspects the problem is one of host and parasite, with the host supplying the mechanism through which the desired result may be accomplished; it is this feature of the reaction with which we are least able to reckon.

The extent of these experiments does not permit of deductions as to the permanency of any of the acquired properties of *Trypanosoma lewisi* that have been discussed. The tendency to revert to the natural or stable form of balance was quite evident in all instances as far as my experiments went, but much more extended series would be necessary before an opinion could be expressed as to the impress left upon *Trypanosoma lewisi* by such experimental procedures.

TABLE VIII.

Rapid Passage of Large Doses of Virus in Small Rats.

Trypanosoma lewisi, Strain V, Series 4.

Generation.	No.	Weight in gm.	Dy. of passage.	Incubation period in dys.	Trypanosomes in blood.	Symptoms of infection.	Duration of infection in dys.	Termination of infection.	Remarks.
I	1	73	5th	1	++++	Slight	30+	Survival	Series started from rat 1, series 3.
II	2	96	4th	1	++++	Slight	30+	Survival	Condition good.
III	3	51	2d	1	++++	Moderate	30+	Survival	Infection very light and condition poor at 30 dys.
IV	4	200	2d	1	+++	Marked	22	Death	
V	5	75	1st	1	++++	Slight	30+	Survival	Condition good.
VI	6	62	2d	1	++++	Moderate	30+	Survival	Condition fair.
VII	7	54	2d	1	++++	Moderate	30+	Survival	Condition poor.
VIII	8	92	2d	1	++++	Slight	30+	Survival	Condition good.
IX	9	72	2d	1	++++	Slight	30+	Survival	Condition good.
X	10	68	2d	1	++++	Slight	30+	Survival	Condition good.
XI	11	63	3d	1	+++	Slight	30	Recovery	Condition fair.
XII	12	56	3d	1	+++	Moderate	22	Recovery	Condition poor.
XIII	13	47	3d	1	+++	Moderate	28	Recovery	Late symptoms marked.
XIV	14	38	3d	1	++++	Marked	30+	Survival	Condition very poor.
XV	15	53	4th	1	+++	Moderate	29	Recovery	Condition good.
	16	61	4th	1	+++	Slight	18	Recovery	Condition good.

In conclusion, since the object of the investigation was to obtain indications of the nature and mode of action of the factors influencing the biological properties of *Trypanosoma lewisi,* the results must be interpreted in a broad sense, as much of the detail requires long and careful study. The experiments have served, however, to give a clearer insight into the relations existing between *Trypanosoma lewisi* and its host and afford a basis for formulating a conception of the essential elements of the virulence of this organism. The results also indicate that immunological reactions of two distinct types exercise a dominant influence as determinative factors in natural and experimental modifications of the species, and that by judicious control of these forces marked changes in the biological properties of *Trypanosoma lewisi* may be accomplished.

SUMMARY.

1. Different strains of *Trypanosoma lewisi* represent different states of biological balance, especially between the powers of propagation and resistance to destruction.

2. The biological status of a given strain of *Trypanosoma lewisi* is subject to cyclic variations as the result of immunological reactions in the blood of the host.

3. The factors limiting reproduction and causing destruction of *Trypanosoma lewisi* in the blood are appreciably independent of each other. It is possible, therefore, to influence these processes separately and even in opposite directions.

4. The virulence of *Trypanosoma lewisi,* manifested in its highest form, is dependent upon some degree of reproductive fastness, strong antigenic action, and susceptibility to destruction, varying degrees in the development of these properties producing corresponding variations in the degree of virulence.

5. By a properly regulated system of passage the properties of *Trypanosoma lewisi* that determine its infection cycle and its virulence may be eventually so altered as to change completely both the nature and course of the infection. Such a system of passage must be adapted to the particular strain of *Trypanosoma lewisi* used.

6. Immunological reactions exercise a dominant influence in determining the ultimate biological variations of *Trypanosoma lewisi.*