MORPHOLOGICAL AND DEVELOPMENTAL ANOMALIES OF A PATHOGENIC STRAIN OF TRYPANOSOMA LEWISI AND THEIR RELATION TO ITS VIRULENCE.*

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(From the Laboratories of The Rockefeller Institute for Medical Research. Plates 67 and 68.

The occasional references to unusual manifestations of virulence and the numerous descriptions of morphological and developmental anomalies of *Trypanosoma lewisi* contain scarcely a suggestion of a connection existing between changes in the virulence and changes in the morphology and developmental tendencies of the organism. The first suggestion of the possibility of such a relation is contained in the work of Wendelstadt and Fellmer, on the effect of the passage of *Trypanosoma lewisi* through cold-blooded animals. These authors noted an increase in the virulence of the trypanosome for rats, and, at the same time, a profound alteration in the morphology of the organism, the main feature of which was a marked prolongation of the posterior extremity.

In a recent article I published an account of an unusual increase in the virulence of a pathogenic strain of *Trypanosoma lewisi*. As this strain showed many morphological and developmental peculiarities, the life cycle and morphology of the organism were studied in eighty-two rats, with especial reference to the nature of the infection. In different series of infections, the rate of passage and the dose of trypanosomes were varied in an attempt to modify the resulting infections. As a control to this series, a study was made of a parallel series of rats infected with a typical benign strain of

* Received for publication, April 13, 1914.
Trypanosoma lewisi that showed no morphological or developmental peculiarities. The details of these experiments and the effect of such procedures, as indicated, upon the course of the infection will be presented in another paper.

The usual life cycle and morphology of Trypanosoma lewisi in the rat's blood are so well known that they require no description, and it is not my purpose to present an exhaustive study of the anomalies of this organism and the factors giving rise to them, but rather the relation of anomalous development and morphology to the virulence exhibited by this particular strain. Therefore, only those deviations from the normal will be described that were pronounced and occurred with sufficient frequency in both fresh and stained preparations to be regarded as characteristic of the strain or of a particular infection. Most, if not all, of the peculiarities of this strain have been recognized by other workers but some of them are comparatively rare and apparently have not all been recognized in a single strain, while the conditions giving rise to these anomalies are but little understood.

DEVELOPMENTAL ANOMALIES.

In addition to the usual forms of multiplication, two clearly differentiated types of longitudinal division were observed; both of these were examples of equal binary fission; in rare instances the division was unequal or was multiple. The first of these types of division (figures 1 and 2) is more closely related to the usual process of multiplication as it occurred in trypanosomes showing the increased size, granular and basophilic cytoplasm characteristic of this period in the life cycle of the organism. Such forms of division were never numerous and were observed only during the usual period of multiplication. The sequence in the process of division could not be determined with certainty, as our only means of recognizing these forms comes with the division of the flagellum and undulating membrane. It is certain that division of these structures may occur first, but it is impossible to say whether trypanosomes with a divided nucleus or blepharoplast may not ultimately divide in this manner.
Anomalies of Pathogenic Strain of Trypanosoma lewisi.

The second type of longitudinal division (figures 3 to 7) occurred far more frequently than the first and was observed during all phases of the blood infection. These forms presented a great variety of sizes, occurring in organisms that were quite large as well as in exceedingly small ones, but the general contour, nature of the cytoplasm, and staining reactions of all were characteristic of adult trypanosomes. A study of a large series of these forms indicated that the usual sequence of division was flagellum, undulating membrane, nucleus, and blepharoplast; but again, the sequence was undoubtedly subject to variation.

Several examples of multiple longitudinal division of adult trypanosomes were seen, one of which is shown in figure 7, but unequal division of this type of organism was not observed.

From published accounts of longitudinal division of Trypanosoma lewisi, it is uncertain whether both of these types of division have been recognized; the second is undoubtedly the one that is generally described. In a sense, the two types might be regarded as but one, since they grade into each other and probably represent a common tendency initiated in the young trypanosome and persisting in the adult organism even after all other forms of multiplication have disappeared from the blood.

The almost endless variety of forms exhibited by Trypanosoma lewisi in the usual process of multiplication precludes any consideration of the morphology of such organisms except as regards the degree of irregularity. Further, during the period of multiplication variations in the rate, extent, and persistence of multiplication may occur which markedly alter the character of the infection. At least two types of deviation from the normal have been recognized as indicating an increased severity of infection; either multiplication may be very rapid and marked with great numbers of irregular forms, especially small and imperfectly developed trypanosomes, or, with a normal rate and type of multiplication, this period of the life cycle does not cease at the usual time but continues on through the second or even the third week of the infection. According to our experience, the first type of infection is typically acute and occasionally terminates fatally at the height of multiplication, while the second, although usually more severe, progresses more slowly, and
fatal terminations occur, as a rule, after the second week of the infection.

ATYPICAL MORPHOLOGY.

Adult forms of *Trypanosoma lewisi* are generally uniform in character. The variations that we noted concerned the size, the nature of the flagellum and undulating membrane, the character of the posterior extremity, and the nucleus and the blepharoplast of the trypanosome.

Extremely small trypanosomes, measuring no more than 7 to 8 micra from the tip of the posterior extremity to the origin of the free flagellum, but possessing all the other characteristics of the normal adult trypanosome, were numerous in some severe infections. These small trypanosomes (figures 8 and 9) are of especial interest, as they appear to be identical with the small forms regarded by Swellengrebel and Strickland as peculiar to the life cycle of *Trypanosoma lewisi* in the invertebrate host.

The posterior elongated forms of *Trypanosoma lewisi* described by many authors, and erroneously regarded by some as "of constant occurrence and very numerous at a certain stage of the multiplication-period," were very numerous in this strain of the organism. In some instances 10 per cent. of the trypanosomes showed this peculiarity (figure 10). Two types of elongated extremity were observed with about equal frequency. One of these possessed a delicately pointed tip (figures 11 and 12), and the other a bulbous extremity (figures 3 and 13). These posterior elongated forms were observed during all periods of the infection, but were most numerous during the latter part and immediately following the multiplication period. While this variety of trypanosome was usually larger than the normal adult, extremely small and irregular forms, such as those in figures 14 and 15, were occasionally seen. Other peculiarities of structure that usually accompanied an elongation of the posterior extremity were a highly developed and plicated undulating membrane and a short free flagellum, both of which are shown typically in figure 11. In a few fortunately stained prepa-

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Anomalies of Pathogenic Strain of *Trypanosoma lewisi*.

Rations of these organisms a series of extremely faint longitudinal striations were seen that suggested the presence of myonemes.

Trypanosomes with an abnormally short posterior extremity (figure 16) were not infrequently observed along with the elongated variety. Occasionally there was a virtual absence of the post-blepharoplastic segment, the blepharoplast being practically at the tip of the extremity.

The undulating membrane and the flagellum of *Trypanosoma lewisi* may vary quite independently of the posterior extremity. The two trypanosomes in figure 17 show extremes in the development of the undulating membrane, while figure 18 shows an organism in an early stage of division with an unusually prominent membrane. Figure 19 illustrates an extreme shortening of the free flagellum in an otherwise peculiar trypanosome.

Innumerable irregularities of the nucleus and blepharoplast have been described in detail by various authors and most of them are properly regarded as involution or degeneration phenomena. I observed total absence of a stainable nucleus, resulting from atypical division, in a few instances. This anomaly occurred in young trypanosomes with no evidence of degeneration, as well as in adult organisms where degeneration could not be excluded.

The production of ablepharoplastic trypanosomes through the agency of drugs has attracted so much attention that the spontaneous occurrence of this anomaly in *Trypanosoma lewisi* deserves especial mention. Figures 20 to 22 show ablepharoplastic trypanosomes that are either young or early multiplication forms with no evidence of degeneration or mechanical distortion. Absence of a blepharoplast was noted in all types of *Trypanosoma lewisi*, but was most frequent in the adult organism. While in most instances there was not the slightest suggestion of a blepharoplast, occasionally there was an extremely small granule (figure 20), representing a rudimentary blepharoplast. Although this type of organism was constant, and even numerous in many infections, it could not be found in other infections of the same strain of *Trypanosoma lewisi*. Examples of the developmental and morphological anomalies of this strain might be greatly extended, but these are sufficient to show the unusual character of the strain.
RELATION OF DEVELOPMENTAL AND MORPHOLOGICAL ANOMALIES TO VIRULENCE.

In studying these features of the organism with reference to the character of the infection, as indicated earlier in this paper, certain facts were brought out that indicated a degree of correspondence between the occurrence of developmental and morphological anomalies and the virulence of the strains with which I was working. As the blood of each of the eighty-two rats infected with our pathogenic strain of *Trypanosoma lewisi* showed most of the anomalies of development and morphology that have been described, these features may be regarded as characteristic of the strain. The control strain which, when first isolated, produced very benign infections and showed no anomalies, subsequently showed an increased virulence in certain series of infections coincident with the appearance of anomalous forms of trypanosomes in the blood of infected rats. The simultaneous alteration of these several properties of the organism suggests something more definite than a mere coincidence.

Further, in both strains the relative numbers and the variety of atypical trypanosomes differed in different infections and were greatest in two classes of infections, both of which have already been described as atypical infections. In one of these the incubation period was short, multiplication was rapid and pronounced, and there were enormous numbers of trypanosomes in the peripheral circulation. Some of these infections were unusually severe and occasionally terminated fatally, while others, in which multiplication ceased early and abruptly, showed a very mild course throughout.

The second class of infections in which anomalies were prominent differed from the first in that the evolution of the infection was more gradual and the period of multiplication persistent. The prominent features of these infections were the persistence of multiplication and the severity of the infection.

In conclusion, then, while it was certain that an intimate relation existed between the developmental tendencies and the morphology of these strains of *Trypanosoma lewisi*, it could not be definitely determined to what degree these characteristics were coordinated with the virulence. There were some facts that might lead one to believe
that as virulence influenced the course of the infections it also exercised an influence upon the developmental tendencies and hence the morphology of the organisms. My observations, however, inclined me to the opinion that, in so far as Trypanosoma lewisi was concerned, developmental tendencies exercised the dominant influence upon the morphology of the organism, and, while these characteristics did not correspond rigidly with the virulence of the organism, that anomalies of development and morphology, to some degree, corresponded with the virulence as manifested in the infections of Trypanosoma lewisi that I studied.

**SUMMARY.**

1. The morphological and developmental anomalies of a pathogenic strain of Trypanosoma lewisi have been described to show the unusual character of the strain.
2. Especial attention is called to the spontaneous occurrence of ablepharoplastic forms of Trypanosoma lewisi.
3. It is pointed out that morphological anomalies were most pronounced in infections that showed unusual conditions of multiplication, and that such infections usually proved severe.
4. Finally, an appreciable correlation between the morphological and developmental characteristics and the virulence as manifested in these examples of infection with Trypanosoma lewisi has been suggested.

**EXPLANATION OF PLATES.**

PLATE 67.

The photomicrographs show a magnification of 1,000 diameters, except figure 10, in which the magnification is 1,050. Figures 1, 3, 5, 7, 10, 16, 18, 21, and 22 are from smears of rat blood stained with Wright's blood stain. All the other figures are from similar preparations stained with Giemsa stain. The figures are not retouched.

**FIGS. 1 to 7.** Longitudinal division of Trypanosoma lewisi. Figures 1 and 2 show trypanosomes with the usual cytoplasmic characteristics of organisms in process of multiplication, while figures 3 to 7 represent division of older forms. Figure 3 shows division of the flagellum in a trypanosome with an elongated posterior extremity, and figure 7 multiple division of an adult trypanosome. **FIGS. 8 and 9.** Small trypanosomes with the characteristics of the adult organism. **FIGS. 10 to 15.** Trypanosomes with an elongated posterior extremity.
(Brown: Anomalies of Pathogenic Strain of Trypanosoma lewisi.)
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Fig. 10. Three adult trypanosomes with posterior elongations. Compare these with the two young forms, also with elongated posterior extremities, and with the normal adult trypanosomes.

Fig. 11. A very large trypanosome with a delicately pointed posterior extremity and a highly developed undulating membrane.

Plate 68.

Fig. 12. An extreme elongation of the posterior extremity.
Fig. 13. Posterior extremity with a bulbous tip. Compare with figure 3.
Fig. 14. Small anuclear trypanosome.
Fig. 15. A young trypanosome with posterior elongation.
Fig. 16. Short posterior extremity in an adult trypanosome.
Figs. 17 to 19. Trypanosomes with highly developed undulating membrane. Compare with figures 11 and 13.

Fig. 20. Trypanosoma lewisi with a rudimentary blepharoplast. Only the dot at the root of the flagellum represents the blepharoplast. The dark line is the result of heavy staining of the flagellum crossing the body of the organism. Compare with figure 11.
Fig. 21. An ablepharoplast trypanosome.
Fig. 22. An ablepharoplast trypanosome with an elongated posterior extremity.