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On the Reacquisition of Virulence in Trypanosomes of the *Brucei*-group.*

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As is well known, trypanosomes of the *brucei*-group undergo morphological and physiological changes during their cycle in the tsetse fly. While they stay in the intra- and extraperitrophic space of the midgut they turn into the slender midgut-forms and become avirulent. Later, after having reached the proventriculus of the fly, they assume the characteristic shape of the still avirulent crithidia. The last part of this complicated migration leads them forward, then backward through the proboscis to the posterior end of the salivary glands. It is here that the transformation into infective metacyclic trypanosomes takes place; and then, when injected with the fly's saliva under the skin of a mammalian host, these metacyclic trypanosomes evolve into the polymorphic blood stream forms. This raises the question whether these changes are due to substances produced in the midgut and salivary gland environment. Findings by WILLIAMSON 1956, WEINMAN and GEIGY 1959, have in fact shown that substances like inositol, arabinose, alanine, taurine and trehalose occur in biologically interesting concentrations in *Glossina palpalis* and *morsitans* respectively, mainly in salivary glands and saliva. This stimulated our later investigations on which we are reporting now. All strains used had been recently isolated in Tororo (Uganda) and kept in the deep freeze until cultured[3].

We first studied whether there exists an affinity between trypanosomes and certain organs of the tsetse fly and if these organs may physiologically induce infectivity to avirulent culture trypanosomes. One knows that culture forms of the *brucei*-group are considered as avirulent and are generally referred to as "midgut

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* This publication is dedicated with great respect to Professor Albert Dubois as it has been promised to him by R. Geigy on the occasion of his 75th birthday (see Liber Jubilaris Albert Dubois, 1963).

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[2] Sincere thanks are expressed to Prof. R. Geigy, Director of the Swiss Tropical Institute, Basle, for making available to me laboratory facilities while I was on sabbatical leave from Pomona College.

[3] For details see GEIGY, R. & M. KAUFFMANN (1964), p. 170. Tororo strains used were *T. rhodesiense:* 115, 117, 118, *T. brucei:* 110, 207, 457.

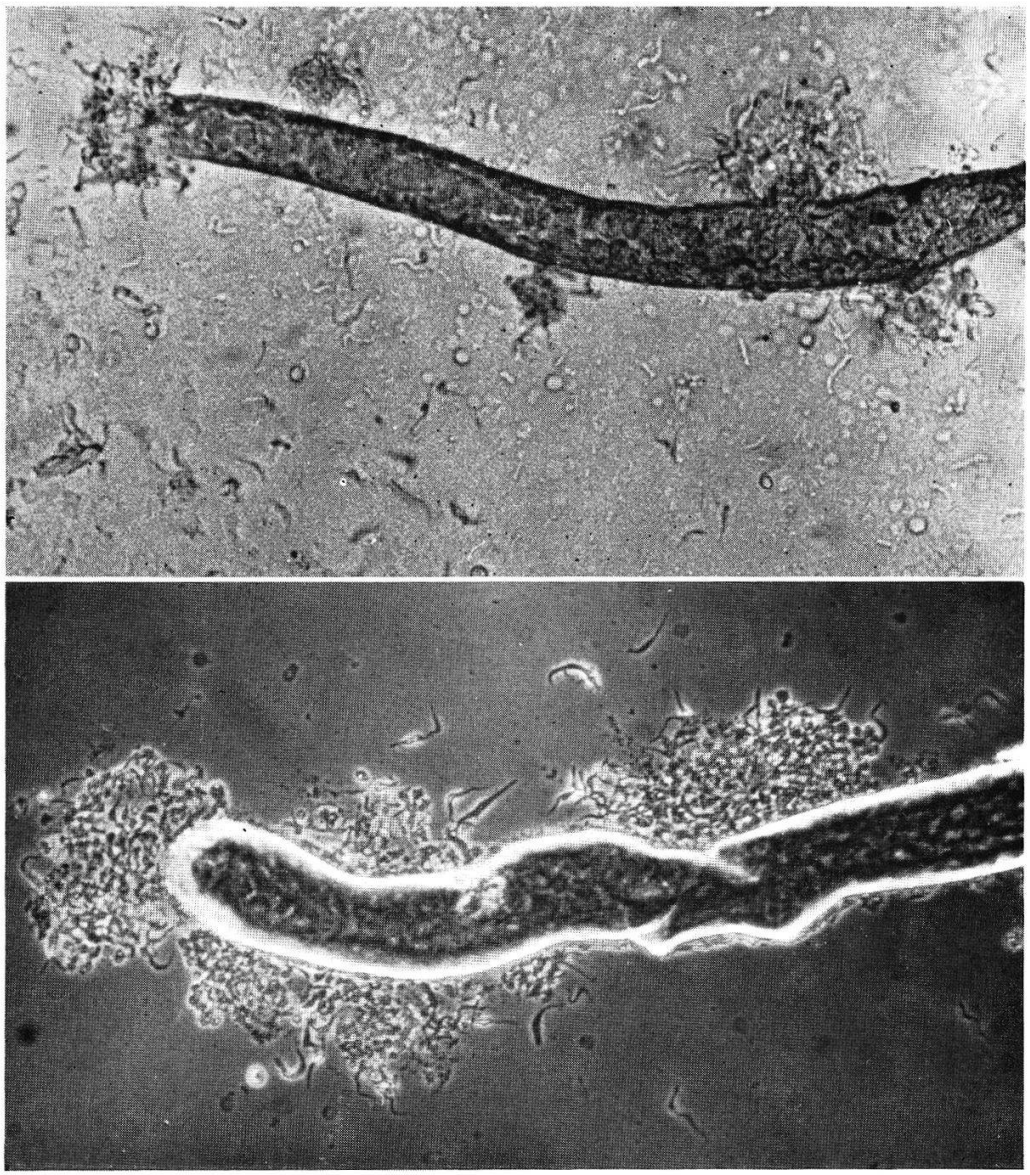

Fig. 1. Culture trypanosomes of *T. rhodesiense* affixed to salivary gland fragments of *G. morsitans* (× 200).

forms" in comparing them with the forms found in the tsetse gut[4]. When we maintained culture forms of the *brucei*-group in T.C. 199 in presence of various isolated tsetse tissues they showed a striking

[4] In doing so one must be aware that the midgut of an infected tsetse fly contains in its intra- and extraperitrophic space exclusively these typical slender forms (there called midgut forms) and that the crithidias and the leishmania-like rounded bodies appear only in the proventriculus, when the midgut forms have already moved forward and passed through the soft part of the peritrophic membrane (cf. TAYLOR, 1932). But culture forms of the *brucei*-group are in reality composed of a mixture of slender midgut forms, crithidias and all sort of morphological transitions between trypanosomal and leishmanial bodies with very short or without flagella. Therefore, beside the metacyclic infectious trypanosomes appearing finally in the lower part of the salivary glands, the organs of the tsetse fly harbour practically the same variety of forms which are found in a haemoculture.

preference for midgut, salivary glands and muscles. No specific organotropism, however, was observed for Malpighian tubes, gonads and fat cells. These results were obtained with organs of *Glossina morsitans, pallidipes* and *brevipalpis* in experiments involving 23 series comprising 160 cultures without counting the numerous controls.

The microphotos taken on the 3rd and 4th day after preparation show culture trypanosomes of *T. rhodesiense* affixed on and assembled around fragments of tsetse salivary glands. They concentrated preferably on broken parts of the gland wall, moved actively and showed many divisions (cf. TRAGER 1959, p. 481). These slide-cultures with trypanosomes affixed to attracting organs declined after 6 to 7 days. When they were injected into mice in between the 3rd and the 7th day, in order to ascertain if virulence was restored, not a single case proved to be positive.

We then tried additions of various substances to *brucei*-group trypanosomes—1st to 25th passage—on Weinman medium at 25°C. First the action of expressed salivary gland juice from *G. morsitans* was investigated. Sterile juice was obtained from freshly isolated and triturated organs in passing it through a Milipore filter. A total of 1,750 glands were used in batches of 5, 10, 20, 50, 70 and 100. When such juice was repeatedly added to *brucei* cultures and the trypanosomes were injected a week later, results proved inconclusive. Only 1 of 42 treated cultures, but to our surprise also 2 of 14 control cultures, became virulent for mice.

More detailed trials were run with single chemical components of salivary glands. Commercially available pure alanine, taurine, arabinose and inositol were added in various ways to *brucei*-group cultures. From among the first three substances only arabinose gave a single case of restoration of virulence. But it was striking that in two series treated with inositol, 3 of 13 and 5 of 8 became virulent. They had all been injected into mice on the 18th day. All controls remained negative.

Despite the results reported by NOVY and MCNEAL 1904 and BEHRENS 1914 who first kept trypanosomes in haemocultures and apparently did not observe complete loss of virulence, even over some years, it became the generally held opinion of investigators in this field that blood culture forms lose their infectivity for mammals. Impressed by the fact that also in our experiments some control cultures became virulent, and encouraged by inspiring discussions Geigy had at the East African Trypanosomiasis Research Organization (EATRO) at Tororo (Uganda), we decided to conduct a systematic study of possible restoration of virulence in *T. brucei* cultures. For this purpose we prepared two large series each of

60 control cultures and 60 cultures containing inositol, alanine, taurine and arabinose. Trypanosome suspensions from 5 of these cultures were injected every other day into mice during the whole life span of our cultures from the 2nd to the 27th day. Possible trypanosome infection was then searched for in mouse tail blood over a period of 8 weeks by means of fresh as well as Giemsa-stained preparations made at the same time.

While one of these large series involving 120 cultures in all did not produce a single infection in the mice, the other exhibited a surprising amount of restored virulence. 15 of 60 control cultures, and 1 of 60 treated cultures proved positive for infectivity to mice. No infectivity could be observed in cultures less than 8 days of age *in vitro*. After a few sporadic cases of virulence from 8-day-old cultures on, a notable peak was reached with cultures injected around the 18th day, followed by a rapid decline in infectivity with cultures older than 23 days.

The results thus obtained showed clearly that it is not the addition of substances but the age of a culture which must be responsible for this restoration. In order to elucidate this point further, we prepared another series of 80 control cultures. 10 of these cultures were injected daily into mice after the cultures had reached 17 days of age and up to the 24th day. Again infectivity was produced in a marked measure, with a peak for 18-day-old cultures. This underlined once more the importance of the age of a culture, but the astonishing fact remained that on our Weinman medium, a series may either show a pronounced peak for infectivity around the 18th day, or remain completely negative. In our experiments, out of a total of 36 separate series, 17 series remained totally uninfective [5]. What is the explanation for such negative series?

As the only variant in our cultures was the blood component of the medium, we decided to investigate this particular factor in some more details. Using the blood of 5 different human donors, 30 media from each donor were prepared at the same time. Three days, three weeks, and six weeks after preparation, 10 media of each group were inoculated, each time with one suspension. This was done to see whether storage of sterile culture medium at 4°C for varying lengths of time had any effect on subsequently inoculated trypanosome cultures. From these 18-day-old cultures mice were then injected.

The results varied considerably. Cultures from media of donor No. 3 remained always negative with respect to virulence to mammals. Cultures from media of donors 2 and 4 proved always virulent

[5] The 36 series were composed of a total of 803 cultures and the 19 positive series contained a total of 83 positive cultures, that is 10%.

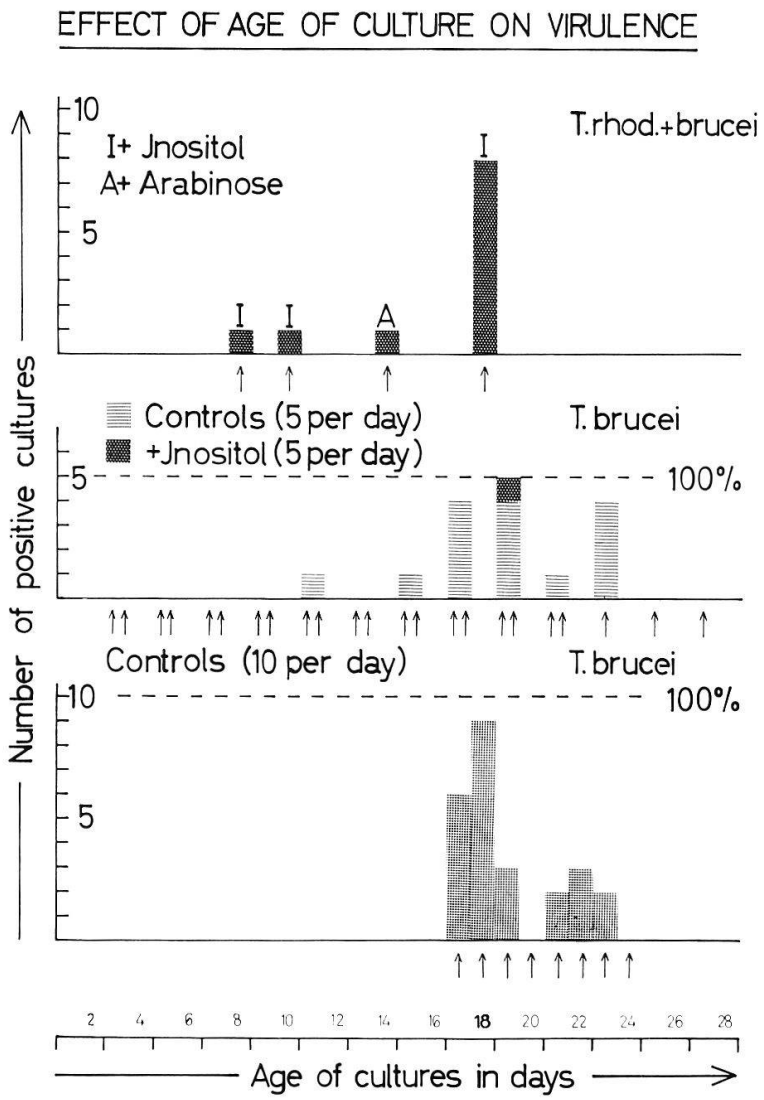

Fig. 2.

with a frequency of 20-50%, whether the media were three days, three weeks, or six weeks old before being inoculated. Surprisingly enough, cultures from media of donors 1 and 5 gave a positive result only in media stored for 6 weeks at 4°C prior to inoculation. While blood from donor 5 produced cultures showing only 10% revival of virulence, all cultures grown on six-week-old medium made with blood from donor 1 became virulent. They also exhibited a much shorter incubation period in the mice than any of the other cultures (Fig. 3). In general series which exhibited a high percentage of infective organisms produced shorter incubation periods in mice and heightened and more virulent parasitaemias (Fig. 4).

As demonstrated in Fig. 4 injections made with cultures from a suitable donor and administred to mice around the 18th day produced not only a high percentage of positives, but also in more

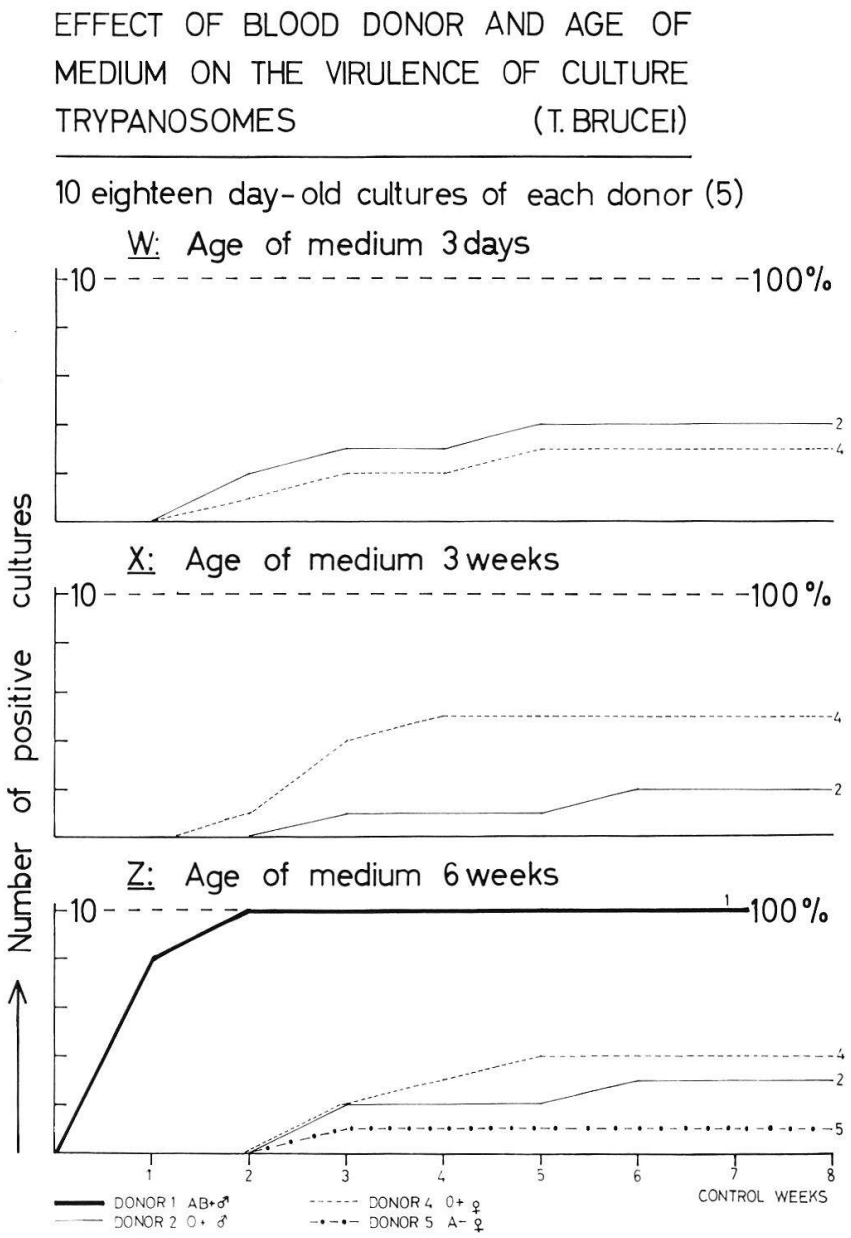

Fig. 3.

cases, where two mice were injected from a single culture, both developed parasitaemia. Where only a low percentage of infectivity was realised, parasitaemia was so low that trypanosomes could be demonstrated only occasionally (see Table 2). Also mice showing such low infections survived for 3 months and more, while in the case of the 100% positive series (see Table 1) one mouse died already after 17 days, and only 1 of 20 survived 8 weeks. All this seems to indicate that the number of metacyclic forms must vary considerably in these cultures and probably reaches a peak around the 18th day. No clear-cut evidence for an increase in the number for metacyclic trypanosomes in cultures, 16 to 23 days of age, could

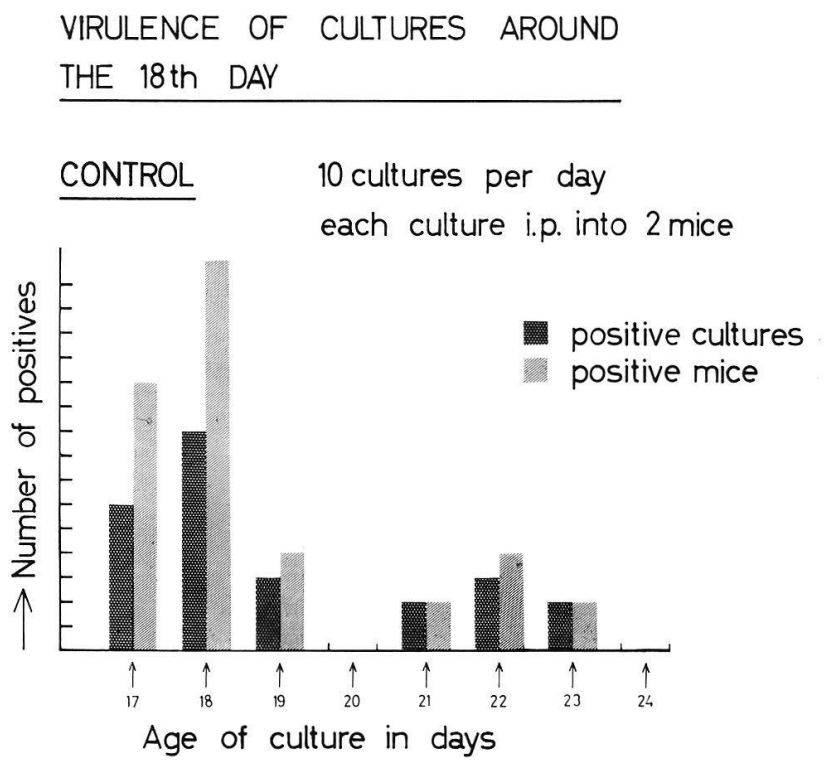

Fig. 4.

be obtained by examination of Giemsa-stained slides prepared from culture fluid. The number of trypanosomes, morphologically appearing as metacyclic forms, is so low, even in slides of 18-day-old cultures, that a valid comparison with the number discernible in cultures of different ages cannot be made.

As to the reacquisition of virulence in trypanosomes of the *brucei*-group the conclusions which can be drawn from all our results are the following:

1. The age of the culture seems to be of major importance; in the case of Weinman media the optimum is about 18 days.

2. Also decisive is the individual blood donor; there are more or less suitable as well as completely unsuitable donors.

3. The length of storage of the media before inoculation can also be important; blood from excellent donors may reveal its properties only after prolonged storage before being inoculated.

4. Perhaps one can assume that these same three factors combined may also play a major role in the trypanosome cycle which takes place in the tsetse fly: First it is remarkable that this cycle —from the uptake of the infective blood meal until the presence of metacyclic trypanosomes in the salivary glands—takes about the same time as the one we have demonstrated in the cultures. Secondly the astonishingly low (natural and experimental) infection rate typical for the *brucei*-group trypanosomes in the tsetse

TABLE I

Series Z 1-10 all from donor 1: Mouse protocol
Cultures inoculated on the 18th day into two mice each

Culture check before injection	Mouse	Days after infection of mice (ID) 7	14	23	28	36	42	49	58
Z 1 ++ !	Z 1	+++	++(+)	+++ ↟					
	Z 2	+++	++(+)	+++ ↟					
Z 2 +++	Z 3	+++	++(+)	+++ ↟					
	Z 4	(+)	++	+++ ↟					
Z 3 +++	Z 5	++	+(+)	++	+++	† 36. (ID)			
	Z 6	(+)	++(+)	+++	++(+)	+++	+++	† 47. (ID)	
Z 4 ++(+)	Z 7	–	–	–	–	–	–	–	–
	Z 8	–	++(+)	+(+)	++	† 34. (ID)			
Z 5 +++	Z 9	+++	+(+)	+(+)	+++	+++	+++	† 47. (ID)	
	Z 10	+	+++	++	+++	+++	† 38. (ID)		
Z 6 ++ !	Z 11	++++	+	† 17. (ID)					
	Z 12	+(+)	+	++(+)	+++	++++	† 38. (ID)		
Z 7 ++(+)	Z 13	+(+)	++	+++	+++	+	++	† 49. (ID)	
	Z 14	–	–	–	–	–	+	+++	+++
Z 8 +++	Z 15	++(+)	+++(+)	† 18. (ID)					
	Z 16	((+))	+++	++	+++	† 36. (ID)			
Z 9 ++(+)	Z 17	(+)	++	+++(+)	++++	† 32. (ID)			
	Z 18	–	++	+	+	† 36. (ID)			
Z 10 +++ !	Z 19	–	++	++(+)	++	† 35. (ID)			
	Z 20	–	+++	+++	+++	† 35. (ID)			

↟ mouse killed

fly, may also be a result of these factors. In choosing suitable donors for the blood meals which shall infect the fly, this rate may perhaps be raised.

References.

BEHRENS, CHARLES AUGUST. (1914). An attenuated culture of *Trypanosoma brucei*. — J. infect. Dis. *15*, 24-62

GEIGY, R., HUBER, M., WEINMAN, D. & WYATT, G. R. (1959). Demonstration of

TABLE II

Series Z 11-20 all from donor 2: Mouse protocol
Cultures inoculated on the 18th day into two mice each

Culture check before injection	Mouse	Days after infection of mice (ID) 7	14	23	28	36	42	49	58
Z 11	Z 21	—	—	—	—	—	—	—	—
+++(+)	Z 22	—	—	—	—	—	—	—	—
Z 12	Z 23	—	—	—	—	—	—	—	—
++	Z 24	—	—	—	—	—	—	—	—
Z 13	Z 25	—	—	—	—	—	—	—	—
+++	Z 26	—	—	+	—	—	—	—	+
Z 14	Z 27	—	—	—	—	—	—	—	—
+++	Z 28	—	—	—	—	—	(+)	—	+
Z 15	Z 29	—	—	—	—	—	—	—	—
+++	Z 30	—	—	—	—	—	—	—	—
Z 16	Z 31	—	—	+	++	+ ↯			
+++(+)	Z 32	—	—	—	—	—	—	—	—
Z 17 +	poor culture; not injected								
Z 18	Z 35	—	—	—	—	—	†42.(ID)		
+++	Z 36	—	—	—	—	—	—	—	—
Z 19	Z 37	—	—	—	—	—	—	—	—
+++(+)	Z 38	—	—	—	—	—	—	—	—
Z 20	Z 39	—	—	—	—	—	—	—	—
++(+)	Z 40	—	—	—	—	—	—	—	—

↯ mouse killed

trehalose in the vector of African trypanosomiasis: the tsetse fly. — Acta trop. *16*, 255-262

GEIGY, R. & KAUFFMANN, M. (1964). On the effect of substances in Glossina tissues on culture trypanosomes of the *brucei*-subgroup. — Acta trop. *21*, 169-173

LEWIS, E. A. & LANGRIDGE, W. P. (1947). Developmental forms of *Trypanosoma brucei* in the saliva of *Glossina pallidipes* and *Glossina austeni*. — Ann. trop. Med. Parasit. *41*, 6-13

NOVY, FREDERICK, G. & MCNEAL, WARD J. (1904). On the cultivation of *Trypanosoma brucei*. — J. infect. Dis. *1*, 1-30

TAYLOR, A. W. (1932). The development of West African strains of *Trypano-*

soma gambiense in *Glossina tachinoides* under normal laboratory conditions and at raised temperatures. — Parasitology *24*, 401-418

TRAGER, WILLIAM. (1959). Tsetsefly tissue culture and the development of trypanosomes to the infective stage. — Ann. trop. Med. Parasit. *53*, 473-491

WEINMAN, D. (1946). Cultivation of African sleeping sickness trypanosomes on improved, simple, cell-free medium. — Proc. Soc. exp. Biol. Med. (N.Y.) *63*, 456-458

WEINMAN, D. (1957). Cultivation of trypanosomes. — Trans. roy. Soc. trop. Med. Hyg. *51*, 560-561

WILLIAMSON, J. (1956). The composition of tsetse fly saliva. 2. Analysis of amino acids and sugars by paper partition chromatography. — Ann. trop. Med. Parasit. *50*, 334-344

WYSS-HUBER, M., JÄGER, H. & WEISS, E. (1960). Nachweis kleiner Mengen Trehalose und verwandter Zucker in einem Gemisch. — Helv. chim. Acta *43*, 1010-1013

WYSS-HUBER, M., STRIEBEL, H., WEISS, E. & GEIGY, R. (1961). Papierchromatographischer Nachweis verschiedener Zucker und spektro-photometrische Bestimmung des Glykogens in Extrakten von Glossinen, Reduviiden und *Ornithodorus moubata*. — Acta trop. *18*, 46-57

Zusammenfassung.

Es ist in zahlreichen Serien versucht worden, die Virulenz bei Kulturtrypanosomen der *brucei*-Gruppe experimentell wiederherzustellen. Zu diesem Zweck sind lebende Fragmente von Tsetsefliegenorganen, Speicheldrüsenextrakte dieser Fliegen sowie verschiedene chemische Substanzen Trypanosomenkulturen beigegeben worden. Die große Mehrzahl dieser Versuche verlief negativ, lediglich nach Zugabe von Inositol konnte in gewissen Fällen eine Wiederherstellung der Virulenz dieser Erreger gegenüber Mäusen festgestellt werden. Da jedoch außerdem mit vereinzelten unbehandelten Kontrollkulturen bei Mäusen ebenfalls Parasitaemie erzeugt werden konnte, sind in der Folge systematische Untersuchungen angestellt worden über allfällig auftretende Virulenz, vergleichsweise in behandelten und unbehandelten Kulturen. Verabfolgte man täglich vom 2. bis 27. Tag an solche Kulturaufschwemmungen steigenden Alters an Mäuse, so stellte man fest, daß sich in unbehandelten Weinman-Kulturen, noch deutlicher als in behandelten, vom 8. Tag an von selber zunehmende Virulenz einstellte, und zwar mit einem charakteristischen Gipfel bei 18 Tage alten Kulturen.

Der autonom sich abwickelnde Virulenz-Zyklus bei Kulturtrypanosomen der *brucei*-Gruppe hängt aber, wie weiter gezeigt werden konnte, außerdem noch vom Spender des in diesen Kulturen verwendeten menschlichen Blutes ab sowie auch von der Zeitdauer, während welcher die Nährböden bis zur Inokulation gelagert werden.

Es kann vermutet werden, daß der seit langem bekannte Virulenz-Zyklus in der Tsetsefliege — d. h. das von morphologischen Veränderungen begleitete Avirulent-Werden der Trypanosomen in der aufgenommenen Blutmahlzeit und die metazyklische Wiederherstellung der Virulenz in der Speicheldrüse — mindestens bis zu einem bestimmten Grad auch von den hier aufgezeigten drei Faktoren (Kulturalter, Blutspender und Lagerdauer) abhängt und daß der auffallend niedrige Infektionsindex der Tsetsefliege sich zum Teil damit erklären läßt.

Résumé.

On a tenté, au cours de nombreuses séries expérimentales, de restituer leur virulence aux trypanosomes de culture du groupe *brucei*. Pour ce faire, on a ajouté aux cultures de trypanosomes soit des fragments vivants d'organes de mouches tsé-tsé, soit des extraits de leurs glandes salivaires, soit diverses substances chimiques. La grande majorité de ces essais n'ont donné aucun résultat positif, sauf cependant en présence de l'inositol, où certaines séries de trypanosomes traités ont alors présenté une restitution de leur virulence pour la souris blanche. Mais comme l'évolution spontanée d'une parasitémie fut également observée dans certaines des séries témoins de souris blanches, il devenait nécessaire de procéder à une étude systématique et comparative de séries traitées et de séries témoins. Les contrôles des cultures, effectués entre le 2e et 27e jour, ont donné un résultat surprenant : les séries témoins développent, mieux encore que les traitées, une virulence croissante pour la souris blanche ; la parasitémie débute le 8e jour et atteint son sommet caractéristique dans des cultures âgées de 18 jours.

Un cycle autonome de virulence se déroule donc chez des trypanosomes de culture du groupe *brucei*. Des recherches ultérieures ont en outre démontré que ce cycle dépend, d'une part du donneur de sang humain utilisé dans nos cultures Weinman, d'autre part du laps de temps durant lequel on laisse reposer le milieu avant qu'il ne soit inoculé de trypanosomes.

On peut présumer que le cycle évolutif des trypanosomes dans la tsé-tsé — perte de virulence liée aux transformations morphologiques, enfin restitution de cette virulence dans les glandes salivaires — dépend également, dans une certaine mesure, des 3 facteurs dénoncés par nos expériences : âge de la culture, donneur de sang et temps de conservation du médium. Ceci expliquerait peut-être pourquoi la mouche tsé-tsé présente dans la nature, de manière caractéristique, un indice d'infection étonnamment bas.