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Miscellanea.

On the Effect of Substances Found in Glossina Tissues on Culture Trypanosomes of the *Brucei*-subgroup.*

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It has long been known that trypanosomes of the *brucei*-subgroup undergo various morphological and physiological changes after being taken up by the tsetse-fly in its blood meal. The polymorphic blood forms first develop into the elongated midgut forms which have been proved to be avirulent. Still actively dividing, they then pass back into the proventriculus via the extraperitrophic space of the midgut. From there, they must reach the salivary glands which they do by passing over the tip of the proboscis and through the salivary duct. During this complicated migration they turn into crithidial forms through the typical forward dislocation of the kinetoplast. But the virulence is only restored when the crithidias have been transformed into the well known metacyclic trypanosomes. After completion of the whole cycle, those are found in the distal part of the glands and in the salivary duct, ready for transmission. One is tempted to believe that the physically and chemically entirely different media into which the trypanosomes are transferred following their uptake by the tsetse-fly, may be responsible for these changes, particularly also for the loss or restitution of virulence. Therefore many research workers studied this problem in different ways. We like to give here a preliminary report on our own trials and results.

WEINMAN (1957) reported some cases of restitution of infectivity of *T. rhodesiense* cultures through the addition of trehalose, a disaccharide found in many insects. GEIGY et al. (1959, 1960), partly in collaboration with WEINMAN and WYATT started looking for trehalose in extracts of whole tsetse-flies or individual organs. WILLIAMSON (1956) who investigated the composition of tsetse-fly saliva by paper chromatography found considerable amounts of inositol as well as a pentose which he identified as very probably being arabinose. For his experiments, he collected the saliva of *Glossina palpalis* by the "probe" technique after BURTT (1946). GEIGY et al. (1961), while searching for trehalose, were also able to demonstrate high concentrations of arabinose in the salivary glands by chromatographic analysis of extracts.

As culture trypanosomes of the *brucei*-subgroup appear to be morphologically similar to the avirulent forms found in Glossina, and as in nature the trypanosomes of this group regain their infectivity for the mammalian host in the salivary glands exclusively, it seemed indicated to study the influence of the substances mentioned above on culture trypanosomes.

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Material and methods.

We employed three freshly isolated strains of *T. rhodesiense* (EATRO 115, 117 and 118) and one strain of *T. brucei* (EATRO 110). All four strains had been deep frozen in Tororo/Uganda [1] after a few passages on white mice. A few ampoules of each strain were sent to us, kept at —70°C and used one by one as necessary. The material was thawed up rapidly and injected into white mice or rats. As soon as well positive animals were available, the strains were isolated on WEINMAN's original medium (1946, 1961). In all our experiments we used the same monophasic medium, blood agar slopes 8 ml per tube. We added 2000 I.U. Penicillin in 0.2 ml saline to every second primary culture. Five days later, all cultures showed good growth and were free of bacteria. As, however, more trypanosomes were found in the tubes treated with Penicillin than in the untreated ones, we added thenceforward 600 to 1000 I.U. Penicillin to each ml of medium used. The concentrations of the substances studied were calculated in millimols (MM) in order to get comparable results. Trypanosomes from each culture tube were washed out with saline and injected i.p. into young white mice of 17 to 20 grams.

Experimental.

In a first series of experiments, cultures of *T. rhodesiense* strains 115, 117 and 118 were treated either with trehalose or arabinose. A single application from 0.03 to 0.12 MM showed no effect at all. Three consecutive doses of 0.06 MM each added to the same culture, gave a positive result in 1 of 8 cases treated in the same way with arabinose [2]: the mouse inoculated with the corresponding culture (strain 117) showed a few trypanosomes in the peripheral blood after 5½ weeks. Trehalose was completely ineffective.

In a second series thereafter, all culture tubes were first inoculated with *T. rhodesiense* (117 or 118) and then either inositol [3] or arabinose were added 3 to 5 times. After 14 to 20 days, subcultures were made from each tube and the remaining trypanosomes were washed out with saline and inoculated into one mouse. Each subculture was treated with the same substance several times again. The third subcultures gave the first positive result: 12 cultures treated with inositol were injected into 12 mice. About 6 weeks later, trypanosomes could be found in 3 of these animals. All three infections were much heavier than in the first case after treatment with arabinose. In this series arabinose remained without any effect.

As inositol itself is already well known to be a growth factor for yeast and certain other microorganisms, we confined further experiments temporarily to the addition of this substance.

In a third series we tested again the effect of inositol, employing this time a strain of *T. brucei* (110). As this strain developed in our cultures at the same rate as *T. rhodesiense* 117 and 118, we chose the same dosage and time schedule for the treatment of the new cultures as those that had yielded positive results in the last series with *T. rhodesiense,* i.e. 4 consecutive applications per culture tube followed by subinoculation into fresh tubes and inoculation into mice after 18 days. During our experiments it became apparent that inositol ac-

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[1] We are indebted to the staff of the EATRO-station at Tororo/Uganda, who always kindly supplied us with fresh strains and gave us the history of each strain.

[2] L(+) Arabinose, F. Hoffmann-La Roche Ltd.

[3] Fluka Meso-Inositol for bacteriological purposes.

TABLE 1

Series B (1. subculture): Mice controls B 19–26 26. 12. 1963

Culture control before infection	Days after infection of mice (ID)																
	4	29	39	41	43	46	48	50	54	56	60	62	64	67	74	81	88
B 19 ++++		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B 20 +++(+)		–	–	+ !	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)	(+)	+	(+)	(+)
B 21 +++		–	–	–	–	–	–	+	++ !	++ (+)	+++	+++	++ (+)	++ (+)	† ++++ 74 ID		
B 22 +++(+)		–	++	+++	+++ (+)	++++	++++	† 50 ID									
B 23 +++(+)		(+)	† 39 ID														
B 24 +++	† 4 ID																
B 25 +++(+)		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B 26 ++++		(+)	+++	+++ (+)	++++	† 46 ID											

celerated the growth of *T. brucei* in our cultures and that therefore shorter intervals are indicated for this strain. 10 mice, inoculated with material from the first generation of treated cultures, were still negative after 14 weeks. With the first subcultures 8 mice were inoculated. One of these died 4 days later for an unknown reason. In 5 other animals trypanosomes appeared in the peripheral blood after 29 to 50 days, while the remaining 2 mice were still parasite free after 88 days. The course of the infection varied greatly from mouse to mouse. For example, animal B20 showed a very feeble parasitaemia on each of 14 examinations made between the 41st and 88th day of infection (ID). B22 on the other hand, died 10 days after the trypanosomes were first observed in the blood (cf. table).

A further 25 mice inoculated with 2nd and 3rd subcultures remained also negative.

Discussion.

In a few cases, cultures of *T. rhodesiense* EATRO 117 and 118 and of *T. brucei* EATRO 110 could be reactivated through repeated addition of inositol over a prolonged period of time. In the case of *T. brucei,* the first of the treated subculture-series gave quite good results, 5 out of 8 animals became positive, whereas none became infected from 2nd and 3rd subcultures. This may be explained by the fact, that in the case of *T. brucei* the trypanosomes multiplied much faster after application of inositol than was known to happen with *T. rhodesiense* in similar previous experiments. The cultures were therefore already on the decline when injected into the mice. Furthermore, all our experiments were up to now carried out on blood agar slopes without any liquid phase, which means that only a very limited number of the trypanosomes present in the culture came into contact with the substances added after inoculation. We now try to improve conditions by finding a suitable liquid medium for further tests. That similar experiments carried out by WILLIAMSON (1964) showed no positive results, may be partly due to the fact that his test mice were controlled for 4 weeks only, whereas we have observed onset of parasitaemia as late as 50 days after infection.

As we have to spend several months each year in Tanganyika in the vicinity of a tsetse-fly belt, we are taking the opportunity to try out simultaneously the direct action of tsetse-fly tissues and tissue extracts *in vitro* on inactive culture trypanosomes, using the same strains as mentioned above. As the liquid medium used by us so far (TC 199 after short incubation on blood agar slopes) did not allow to maintain the trypanosomes together with living organs of Glossina long enough, we could not yet get any restoration of virulence for the mammalian host. But we were able to observe a distinct tropism towards certain organs, such as salivary glands and midgut. The trypanosomes are attracted by lesions of these organs and show there great activity and intensified multiplication.

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Über die Funde von Pseudacanthotermes in Tanganyika *(Isoptera, Termitidae).**

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Die wichtigsten Arten der afrikanischen Termitengattung *Pseudacanthotermes* (Sjöstedt 1926), welche wie die meisten Macrotermitinen je zwei Soldaten- und Arbeiterformen besitzt, sind *P. militaris* (Hagen 1858) und *P. spiniger* (Sjöstedt 1900). Sie unterscheiden sich anhand der großen Soldaten vor allem durch die Kopfmasse, die für *P. militaris* 4,50—5,20 mm Kopflänge mit Mandibeln und 2,20—2,70 Kopfbreite und für *P. spiniger* 3,70—4,00/1,85—2,25 betragen. Die kleinen Soldaten von *P. militaris* (2,50—2,85/1,20—1,50) besitzen einen ovalen und flachen Kopf, während er bei *P. spiniger* (2,40—2,60/1,10) oben stark gewölbt ist und sich mit fast geraden Seiten nach hinten verschmälert.

Die übrigen *Pseudacanthotermes*-Arten stehen diesen beiden Hauptarten nahe. In der *P. spiniger*-Gruppe wurden die Subspecies *lujae* (Wasmann 1904), *kohli* (Wasmann 1911), *maynei* (Sjöstedt 1926) und die Art *P. unsgaardi* (Sjöstedt 1926) beschrieben, die heute als Synonyme von *P. spiniger* gelten (EMERSON 1928, SNYDER 1949). In der *P. militaris*-Gruppe wurde auf Grund abweichender Kopfmaße der großen Soldaten eine Reihe von Arten aufgestellt, so *P. laticeps* (Sjöstedt 1905), *P. minor* (Sjöstedt 1913), *P. grandiceps* (Sjöstedt 1915) und *P. curticeps* (Sjöstedt 1924), die durch SNYDER 1949 teilweise zu Subspecies *(P. minor)* und Varietäten *(P. laticeps)* reduziert wurden. Neuerdings hält WEIDNER (1956, 1961) den Artcharakter von *P. minor* für berechtigt. Eine Sonderstellung nimmt die von WEIDNER (1962) beschriebene *P. harrisensis* aus dem Sudan ein.

Das Verbreitungsgebiet von *P. militaris* erstreckt sich über die ganze aethiopische Region, im Norden von Guinea und Kenya bis nach Südafrika. Für Tanganyika gab HARRIS (1936) Morogoro, Muheza und Kigoma als Fundorte an, und nach KEMP (1955) ist die Art im Nordosten von Tanganyika auf die

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* Meinem verehrten Lehrer, Herrn Professor Dr. Rudolf Geigy, zum 60. Geburtstag gewidmet.