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Miscellanea

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Influence of Amino Acids on the Development of Infective *Trypanosoma (Trypanozoon) brucei* in Culture[1]

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From investigations dealing with attempts to grow *T. brucei* in culture to the stage infective to the mammalian host, it has been frequently suggested that since *in vivo* (in the tsetse fly) metatrypomastigotes develop in the salivary glands only, the glandular environment probably is required for such metamorphosis.

Experiments have been conducted by GEIGY (AMREIN, GEIGY & KAUFFMANN, 1965) in which tsetse fly salivary gland juice was added to Weinman blood medium *in vitro* in an attempt to enhance the development of infective forms of *T. brucei.* Also, the demonstration that the sugars inositol and trehalose seemed to be present in biologically interesting concentrations in tsetse fly saliva, led GEIGY et al. (GEIGY, HUBER, WEINMAN & WYATT, 1959) to postulate that these sugars when added to culture medium can enhance restitution of infectivity to culture form *T. brucei.* However, a great number of experiments undertaken in order to test the effects of such additions to Weinman medium failed to prove that these substances in fact did enhance the development of trypanosomes infective to mice.

It appeared to us that an investigation of the influence of certain amino acids as demonstrated to be present in tsetse fly saliva by WILLIAMSON (1956) was a worthwhile undertaking. We therefore made up aqueous overlay solutions of all the 16 amino acids together, as shown in the accompanying Table 1 and added this mixture as 1 ml overlays onto the 5 ml Weinman blood agar slants. Following a 5-day period at 4 °C to allow equilibration of the liquid overlays with the solid slants, we inoculated our culture medium with *T. brucei.* Our trypanosome strain was EATRO stabilate Lab 110 originally isolated in East Africa from a naturally infected heifer and having experienced a total of 8 mouse passages since original isolation. Prior to inoculation into our culture medium this stabilate was thawed and injected into a mouse. After our cultures were incubated at 24 °C for 14 to 19 days, we injected 0.5 ml of such trypanosome cultures, containing approximately 60,000 flagellates per ml, intraperitoneally into Swiss mice.

In two separate series of experiments, involving 26 cultures where we used such amino acid overlay media, 61.5 %, i.e. 16 out of the 26 cultures produced *T. brucei* which elicited parasitaemias in our 52 mice, while all 26 control cultures without overlays proved negative in the 52 control mice.

While a result of 61.5 % positive cultures is an impressive increase over 0 % infectivity obtained from our control cultures in these experiments, it must be stressed that if blood from a particularly "suitable" donor is used in our culture medium, if the medium has been stored at 4 °C for the appropriate length of time

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Table 1. Tsetse saliva amino acids

	mg/liter overlay	in saliva (WILLIAMSON)
β-alanine	150	++
L-arginine	70	+
L-asparagine	150	++
DL-aspartic acid	150	++
L-cysteic acid	30	F (faint)
DL-cystine	20	F
DL-glutamic acid	150	++
glycine	250	+++
L-histidine	20	+
DL-isoleucine	40	+
DL-methionine	30	F
DL-serine methyl ester	60	+
taurine	60	+
DL-threonine	60	+
L-tyrosine	40	F
DL-valine	50	+

Table 2. 14 Essential amino acids

	mg/liter overlay
L-arginine	70
DL-cystine	20
DL-glutamic acid	150
L-histidine	20
DL-isoleucine	40
DL-leucine	120
L-lysine	70
DL-methionine	30
DL-phenylalanine	50
L-proline	40
DL-threonine	60
DL-tryptophan	20
L-tyrosine	40
DL-valine	50

before inoculation with trypanosomes, and if *T. brucei* cultures between 16 and 20 days of age are used, then even such preparations without additions to the medium may yield cultures, all of which contain infective trypanosomes. But all these conditions must be met; and since in the above experiments all our control cultures containing the same blood remained non-infective, the addition of amino acids seems to have been responsible for the restoration of infectivity to our experimental cultures.

We also tested in a like manner the effects of amino acid overlays in which we used a solution containing all the 14 essential amino acids as given for Parker's Medium 199, shown in Table 2. In these experiments 25 % of our cultures proved

infective, while all controls remained non-infective. The dissimilar results obtained for these two kinds of amino acid solution overlays seem to be due to the difference in amino acid composition of tsetse fly saliva and Parker medium 199. We are now testing the effect on *T. brucei* of different combinations and concentrations of these amino acids as well as optimal time of addition to our cultures.

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