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Lack of Incorporation of Tritiated Thymidine in *Trypanosoma vivax in vitro*

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In order to determine some of the characteristics of the cell cycle and *in vitro* metabolism of *T. vivax* pathogenic in ruminants, we designed labeling experiments with tritiated thymidine (^{3}H-thymidine) for preparation of autoradiographs and scintillation counting. Our methods were those routinely used by cytologists with mammalian cells, but included relatively large doses of thymidine (up to 15 μCi/ml medium) as described for other species of trypanosomes: *T. musculi* (VIENS & TARGETT, 1972), *T. brucei* (BALBER, 1971), *T. evansi* and *T. gambiense* (INOKI & TADASUKE, 1969), and *T. mega* (STEINERT & STEINERT, 1962). The results were disappointing as background was high, no specific label was apparent, and scintillation counts were low.

By using *T. brucei* passaged in rats, we were able to obtain large numbers of trypanosomes to perfect our experimental system. Under the conditions available to us, we found the most important factors in obtaining high specific label (Fig. 1)

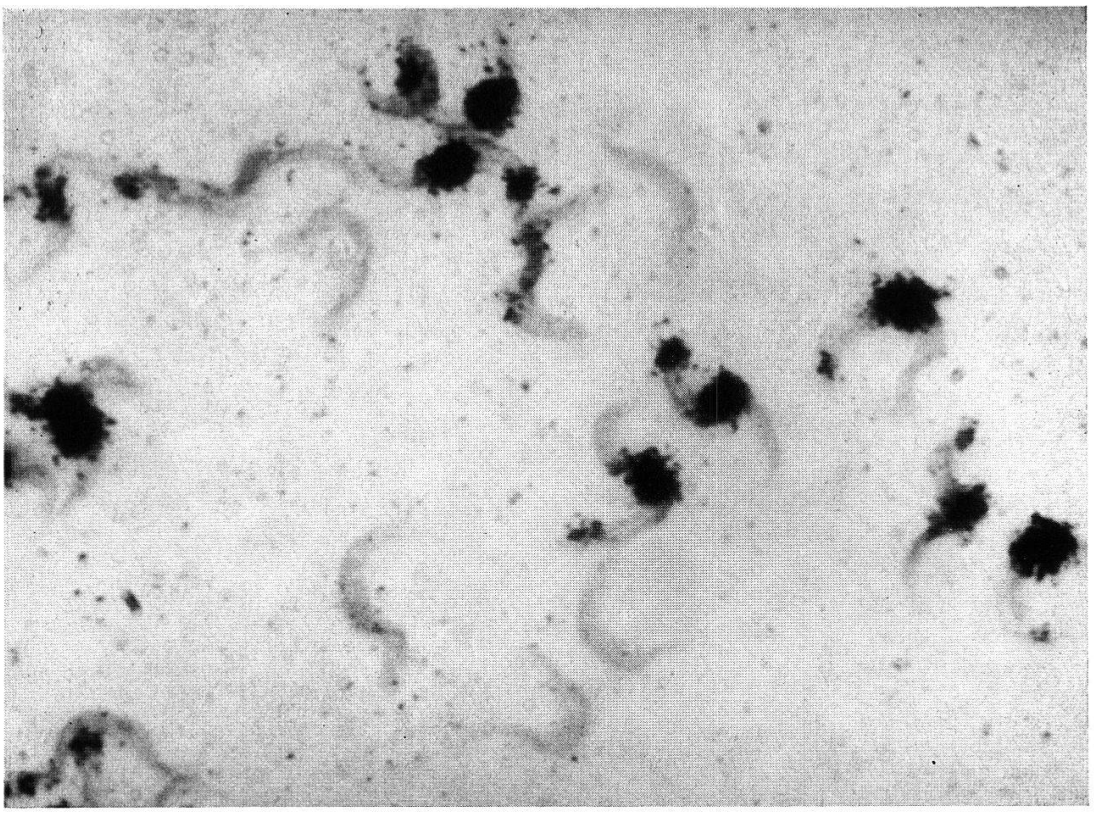

Fig. 1. T. brucei labeled with 15 μCi/ml ^{3}H-thymidine in Hanks saline for 2 hours. Fixed slides were mounted with Kodak (Rochester, New York, U.S.A.) AR-10 stripping film for 4 weeks.

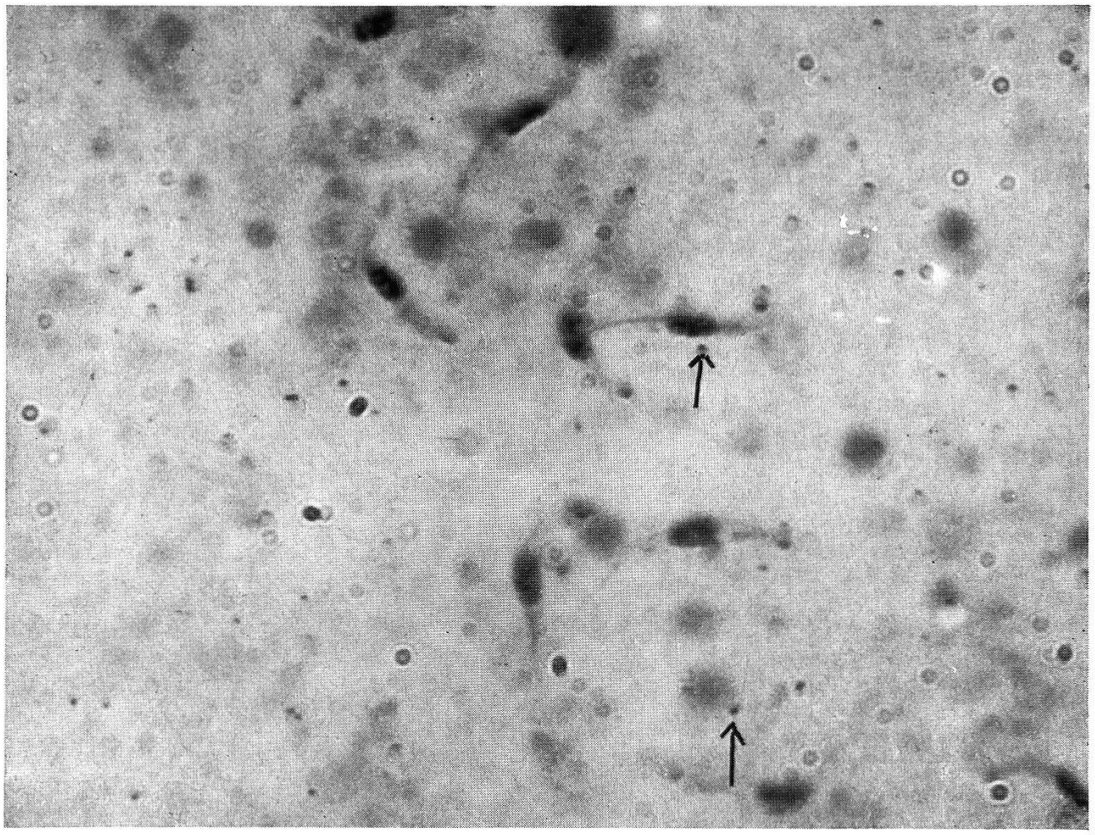

Fig. 2. T. vivax labeled with 25 μCi/ml ^{3}H-thymidine in Hanks saline for 8 hours. Fixed slides were dipped in 1 % PPO-toluene before mounting with stripping film. There is no specific label; a few background grains are apparent (arrow).

were the use of large amounts of high specific activity ^{3}H-thymidine (Amersham, Buckingshamshire, England: 15 μCi/ml, Sp. Act. 26 Ci/mmol) with correspondingly low amounts of thymidine in the suspending medium, and the control of humidity. We were able to control humidity during incubation by the extensive use of silica gel and improved air conditioning. Four weeks incubation of the mounted autoradiographs was adequate. The use of scintillation autoradiography (BALBER, 1971) was not necessary, and when used, increased the scatter of the grains. The high doses of tritium were not toxic to the cells, as survival (counts of organisms using haemocytometer) of the trypanosomes was similar in labeled and unlabeled cultures. Scintillation counts of solubilized (NCS^{TM} Solubilizer, Amersham-Searle, Illinois, U.S.A.) trypanosomes were adequate (Table 1, A–D) for studies of comparative metabolism with different culture conditions (ISOUN & ISOUN, 1974a).

By using the improvements described, we set up experiments with *T. vivax:* trypanosomes were incubated in Hanks saline with 5 % homologous serum and 15–24 μCi/ml ^{3}H-thymidine for two to eight hours. In no autoradiographs was specific label incorporated (Fig. 2). Scintillation counts of solubilized *T. vivax* (Table 1 F and G) were insignificant as compared to controls (Table 1, E and H). The lack of incorporation of ^{3}H-thymidine by *T. vivax* is not indicative of sluggish

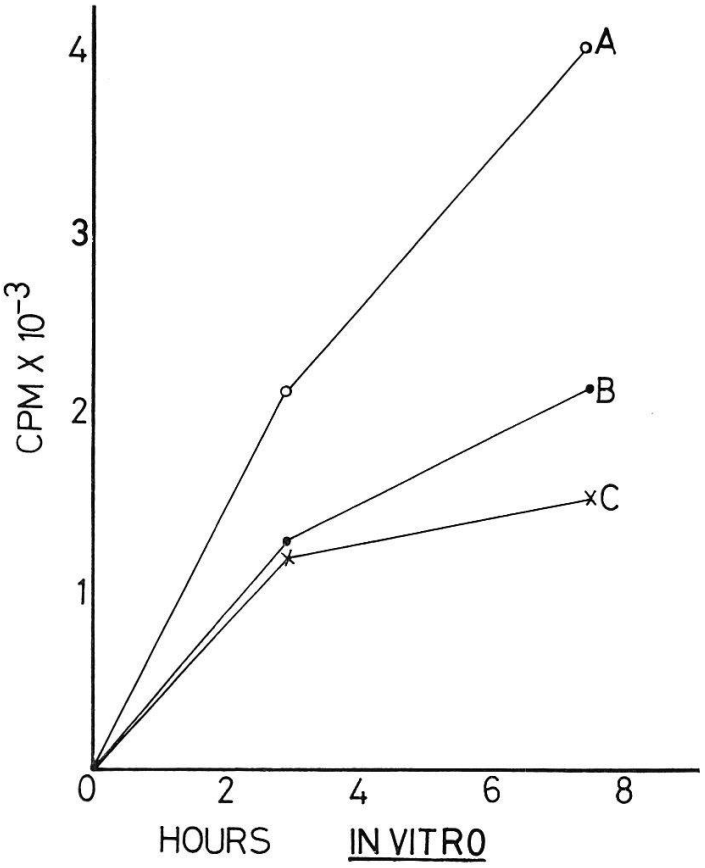

Fig. 3. Effect of foetal calf serum and homologous serum as compared to a saline control on protein synthesis in *T. vivax in vitro* at 25 °. The incubation mixture contained 3 ml saline, 3 μCi ^{14}C-amino acids (10 mCi/mmol), 0.72×10^6 trypanosomes, and 0.5 ml variable: A, homologous serum, B, foetal calf serum, and C, Hanks saline. Trypanosomes were prepared for scintillation counting as described in Table 1.

metabolism of dying organisms, as there was evidence of growth of trypanosomes over the first 24 hours *in vitro* (ISOUN & ISOUN, 1974b), and incorporation of ^{14}C-labeled amino acids (Amersham Buckinghamshire, England) continued during this period *in vitro* (Fig. 3).

The failure to incorporate ^{3}H-thymidine into the DNA of *T. vivax* has not been reported; indeed we are not aware of the inability of other Trypanosomatidae except *Crithidia fasciculata* (GUTTERIDGE & AL CHALABI, 1973) to incorporate exogenous thymidine. *Plasmodium berghei* and *P. vinckei* (WALSH & SHERMAN, 1968), psitticosis organisms (MOULDER, 1966) and *Euglena* (SAGAN, 1965) are reported to be unable to utilize exogenous thymidine. It is logical to deduce that these organisms are able to synthesize their own pyrimidines for DNA synthesis and thus cell division, and multiplication. However, the mechanism by which *T. vivax* is able to synthesize pyrimidines for DNA synthesis is not known. It is tempting to speculate that like the plasmodial group of organisms that synthesize their own pyrimidines, *T. vivax* is capable of fixing carbon dioxide to form carbamyl phosphate, and with aspartic acid, synthesize the pyrimidines. Two enzymes, thymidine kinase and thymidine synthetase, the formation of which depends on the derepression action of the appropriate cistron (PRESCOTT & STONE, 1969), may determine the availability of pyrimidines for DNA synthesis and cell division.

Table 1. Scintillation counts of *T. brucei* and *T. vivax,* isolated from whole blood by erythrocyte lysis (LEEFLANG et al., 1974) followed by DEAE cellulose elution (LANHAM & GODFREY, 1970), and incubated with 15 μCi/ml ^{3}H-thymidine. After incubation, 1 ml of trypanosome suspension was washed 2 × in 10 ml Hanks saline + 5 % foetal calf serum, and resuspended in 0.5 ml saline. 0.2 ml of this suspension was placed in each of two scintillation vials to which 0.7 ml NCS solubilizer was added at room temperature overnight. 5 ml toluene fluor containing 0.4 % PPO (2,5-Diphenyl-oxazole) and 0.005 % POPOP [1,4-bis 2-(5 phenyloxzolyl)-Benzene; phenyloxzolylphenyl-oxazolylphenyl] was added and the vials counted in a liquid scintillation counter (Packard, Model 3101) for 1 minute. The values given below represent averages of the duplicate vials.

Sample	Tryp. count	Species	Time labeled	Medium	Counts/ min
A	*T. brucei*	3.0×10^6	4 hours	saline [1] + 5 % serum [2]	2583
B	*T. brucei*	3.0×10^6	6 hours	saline + 5 % serum	5281
C	*T. brucei*	3.0×10^6	4 hours	199 H [3] + 15 % serum	638
D	*T. brucei*	3.0×10^6	6 hours	199 H + 15 % serum	1117
E	*T. brucei*	3.0×10^6	0	saline + 5 % serum	26
F	*T. vivax*	1.0×10^6	2 hours	saline + 5 % serum	30
G	*T. vivax*	1.0×10^6	8 hours	saline + 5 % serum	32
H	None: background 0				14

[1] Hanks basic salt solution, GIBCO, Grand Island, New York.
[2] Foetal calf serum, GIBCO.
[3] Medium 199 with HEPES buffer, GIBCO.

Additionally *T. vivax* like *C. fasciculata* (GUTTERIDGE & AL CHALABI, 1973) may have, in the presence of exogenous thymidine, high concentration (activity) of the enzyme thymidine phosphorylase (thymidine: orthophosphate deoxyribosyltransferase EC 2.4 2.4) capable of converting tritiated thymidine to thymine – a substance not utilized by the organism. Compounds that are capable of inhibiting enzymes involved in the pathway of the bio-synthesis of pyrimidines in *T. vivax* may serve as chemotherapeutic agents in *T. vivax* infection in ruminants.

In the search for improved media for the *in vitro* cultivation of *T. vivax* it may be found that factors which enhance *de novo* synthesis of pyrimidines in these organisms may also promote growth and multiplication. Currently we are investigating these factors.

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