Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 26 (1969)
Heft: 1

Artikel: Miscellaneum : Suitable blood sources permitting reacquisition of infectivity by culture-form "Trypanosoma (Trypanozoon) brucei"
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DOI: https://doi.org/10.5169/seals-311610

Miscellaneum

Suitable Blood Sources Permitting Reacquisition of Infectivity by Culture-Form *Trypanosoma (Trypanozoon) brucei**

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Introduction

In a previous communication (AMREIN, GEIGY & KAUFFMANN, 1965), it was shown that in order to obtain infective *Trypanosoma (Trypanozoon) brucei* from blood cultures *in vitro,* the individual blood donor, the age of the culture medium prior to inoculation with trypanosomes, and the age of the *T. brucei* population at the time of injection into mice, all play an important role.

Since these results were obtained with blood media incorporating human blood, and since it is stated in the literature that human blood does not readily support growth of *T. brucei,* the resistance of man to infection with *T. brucei* probably being due to the fact that normal human serum kills this trypanosome (HOARE, 1943), we investigated the suitability of blood from a variety of mammals other than man for the *in vitro* production of metacyclic *T. brucei* infective to mice.

Materials and Methods

The strain of *T. brucei* was originally obtained as stabilate Lab. 110 from the East African Trypanosomiasis Research Organization at Tororo, Uganda, and kept deep-frozen until passaged a few times through mice and inoculated into blood cultures. For complete particulars on this strain see BIENZ, 1968.

Weinman blood agar medium (WEINMAN, 1960) was used throughout these investigations, except with some giraffe blood and Calf 82 where Tobie medium (TOBIE et al., 1950) was tried. Penicillin G to give a final concentration of 1000 units per ml was added to the blood media and the blood agar was dispensed in screw-cap test tubes. Freshly made culture tubes were stored for varying lengths of time at 4°C prior to inoculation, and once seeded with trypanosomes, they were incubated at 24°C.

Blood was obtained from several sources as follows: Zoo animals, through the courtesy of Dr. Nathan B. Gale, Assistant Director, Los Angeles Zoo; horse, pig and rabbit, precolostral and newborn calf, as well as some adult bovine blood from Colorado Serum Company, Denver, Colorado; one horse, and several heifer and adult cow samples through the courtesy of Dr. R. H. Packard, California Polytechnic College, Pomona, California. These blood samples were obtained in the citrated, defibrinated, or heparinized form.

Swiss Webster mice (20 g) were purchased from Simonsen Laboratories, Gilroy, California. The mice were injected i.p. with 0.5 ml of 14- to 22-day-old cultures, since previous data had shown that *T. brucei* cultures about 18 days old gave the best results. Mice were checked by tail blood examination for six weeks following injection with trypanosomes.

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* This investigation was supported in part by U.S. Public Health Service Research Grant AI 07451 from the NIAID.

Results

From our screening of blood from a number of different mammals for the preparation of culture media, it soon became apparent that blood from bovids seemed best suited for the production of infective trypanosomes in culture. In that connection it should be recalled that most ungulates which serve as reservoir hosts for *T. brucei* in Africa belong to the family Bovidae. However, the majority of species tested proved to possess blood which allowed development of infective trypanosomes, albeit at a rather low rate.

However, the majority of species tested proved to possess blood which allowed development of infective trypanosomes, albeit at a rather low rate.

Blood utilized from domestic animals other than bovids gave a low percentage of infective cultures. Table 1 shows that among horses, pigs and rabbits, some individual donors were entirely unsuitable, while certain other donors from among the same species were fairly suitable.

Among the few wild animal bloods obtained from the Zoo and used in our culture media as shown in Table 2, it should be noted that three out of 32 cultures made with giraffe blood did produce infective trypanosomes. This is in agreement with Ashcroft's (ASHCROFT, 1959) and Weitz' (WEITZ, 1963) data showing that giraffes in nature are suitable hosts for *T. brucei.*

Culture media made with blood from a Cape Buffalo and from an Aoudad sheep, did not even support sufficient growth *in vitro* to allow injection into mice.

TABLE 1 *Domestic animals other than bovids*

Donor		Medium	Days at 4 °C	Culture age in days Infected cultures / total cultures								
				14	15	16	17	18	19	20	21	22
Horse 90	April 67	W	17		0/10		0/10		**1/10**			
Horse 20	Dec. 67	W defibr.	18				0/6	0/6	0/6			
Horse CP1	June 68	W heparin	18				0/6	**1/6**	**1/6**			
		W defibr.	18				0/4	0/6	0/6			
Pig 47	April 67	W	17		0/4		0/7		0/6			
Pig 48	April 67	W	17	0/10		0/10		0/10		**1/10**		
Rabbit 94	Jan. 68	W	21				0/6	**1/6**	0/6			
Rabbit 95		W	21				0/6	0/6	0/6			
Rabbit 96		W	21				0/6	0/6	0/6			
Rabbit 97		W	21				**2/6**	**2/6**	**2/6**			
Rabbit 94–97 pooled		W	21				0/6	0/6	0/5			

W = Weinman citrated and lysed.

TABLE 2 *Zoo animals*

Donor		Medium	Days at 4 °C	Culture age in days Infected cultures / total cultures								
				14	15	16	17	18	19	20	21	22
Giraffe reticulated	July 67	T washed	21		0/4		0/5		**3/5**			
		T	21		0/6		0/6		0/6			
Elephant	July 68	W	10	0/6		0/6		0/6				
		W not lysed	10	no growth on cultures								
Cape Buffalo	July 67	W	20	no growth on cultures								
Aoudad Sheep	July 67	W	20	no growth on cultures								

W = Weinman citrated and lysed.
T = Tobie.

Table 3 shows the results obtained with blood from members of the Bovidae only. Blood donors ranged from precolostral calves through young calves and heifers, to adult cows. Our best results were obtained with blood from a precolostral calf. But it should be noted that calf, heifer, and adult cow blood also could produce metacyclic trypanosomes and again that certain individuals were entirely unsuited as donors.

Discussion

Our results substantiate a number of observations made earlier (AMREIN, GEIGY & KAUFFMANN, 1965). It seems clear that the blood utilized in the culture medium, being the only variable ingredient, must play a dominant role in permitting development of infective trypanosomes. Our positive and negative results bear this out and emphasize that the individual blood donor, rather than the donor species, is of the utmost importance. There are individuals simply unsuitable as donors, even though the same animal species, sex, and age group can provide suitable blood. Obviously we could never obtain additional blood from precolostral calves; and with one exception, Calf 08 where two separate donations, two months apart, were possible and the blood proved suitable, we were unable to obtain a second donation from the same donor after a certain time interval. Our problem so far has been to locate a suitable animal blood donor and to have it available throughout the year for periodic donations. However, we now have several suitable bovids which may allow us a more systematic analysis of their blood over a prolonged period of time, as well as the designing of parallel experiments using known suitable and unsuitable blood in order to check on inhibition or enhancement of infectivity.

It may be argued that a strain of *T. brucei* kept frozen following isolation and having experienced but a limited number of passages (5–23) might well still be capable of producing metacyclic trypanosomes, while an old laboratory strain will not do this. Obviously an old laboratory strain will have to be tested with suitable donor blood. But the phenomenon nevertheless exists that even a relatively freshly isolated strain of *T. brucei,* capable of producing a certain per-

TABLE 3 *Bovids*

Donor		Medium	Days at 4 °C	Culture age in days 14	15	16	17	18	19	20	21	22
				Infected cultures / total cultures								
Calf 04 precolostral	Sept. 67	W	11				**6/6**	**6/6**	**6/6**			
		W not lysed	11				**3/6**	**3/6**	**3/6**			
		W	61				**2/6**	0/6	0/6			
		W not lysed	61				0/6	**1/6**	0/6			
Calf 55 precolostral	Dec. 67	W	18				0/6	**1/6**	0/6			
		W no plasma	18				0/6	0/6	0/6			
Calf 82 less than 10 days	July 67	W	25	0/5		**1/6**		**1/6**				
		T washed	25	0/6		0/6		0/6				
Calf 44 newborn	May 67	W	23	**1/6**		**4/6**		**1/6**		**2/6**		**1/6**
Calf 22 newborn	Nov. 67	W	17				0/6	0/6	0/6			
		W not lysed	17				0/6	0/6	0/6			
Bovine 08 calf	Dec. 67	W defibr.	18				**1/6**	**3/5**	**1/5**			
second donation	Febr. 68	W	17				**2/6**	**1/6**				
		W defibr.	17				**6/6**	**6/6**				
Bovine 31 calf	April 68	W	12				**1/6**					
		W defibr.	12				0/6					
Bovine 66 calf	June 68	W	18				0/6	**1/6**	**1/6**			
		W defibr.	18				**1/6**	0/6	0/6			
Bovine CP8K1 heifer	Oct. 68	W not lysed	21				**6/6**	**6/6**				
		W defibr.	21				**4/6**	**3/6**	**3/6**			
Bovine CP6 cow	April 68	W	3					0/6				
		W defibr.	3					0/6				
		W	18				**1/6**	0/6	0/6			
		W defibr.	18				0/6	0/6	0/6			

W = Weinman citrated and lysed.
T = Tobie.

centage of infective cultures, will not do so when the culture medium incorporates blood from apparently unsuitable donors, even though the blood of that particular animal species has been shown to be suitable.

Our experiments with the four rabbit blood donors may shed some further light on this. While blood from rabbits 84 and 97 produced infective trypanosomes and blood from rabbits 95 and 96 did not, all four bloods pooled, but otherwise treated in exactly the same way, did not foster development of any infective flagellates.

From this, one is led to speculate that unsuitable blood might well act in an inhibitory fashion, rather than that suitable blood acts by providing a missing factor or substance. This would also explain why precolostral blood, presumably low in antibodies, appears to be the best suited blood.

With this in mind, we ran a series of experiments designed to test whether inactivation of some relatively heat-labile fractions of plasma, the IgG (7S) and possibly some of the IgM (19S) gamma globulins bearing antibodies, might improve the capability of the blood to produce metacyclic trypanosomes. Preliminary results obtained with Weinman blood agar media made by standard methods, but after gelation of the agar exposed for 30 minutes to 60°, 70°, or 80°C respectively, showed that even an inactivation temperature of 70°C for half an hour did not destroy the capacity of the blood to produce infection. But neither did such treatment enhance the infectivity over standard inactivation of 56°C for 30 minutes. Exposure of the medium to 80°C for half an hour seemed to destroy the capacity of the blood to produce infective cultures.

As far as the effects of defibrination or citration of donor blood are concerned, our results are inconclusive. Defibrination appeared to be preferable over citration with some individual donors, while exactly the reverse was true with others. In any case, infective trypanosomes can be grown on blood treated by either method, or with heparin.

During our search for a dependable and suitable donor animal from which blood samples can be taken repeatedly and over a period of time, we already ran several pilot experiments which we hope to undertake now on a larger scale:

a) The relative concentrations of blood cholesterol and uric acid in the blood utilized appeared worthy of further investigation in connection with the development of infective trypanosomes. Since these substances vary over a span of time and with host diet as well as physiological state, it seemed desirable to test these variables. To that end we ran two small series of experiments with cholesteryl linoleate or cholesteryl palmitate added to the culture medium.

The elevated cholesterol level did not render the blood more suitable for metacyclic trypanosomes and a physiologically high uric acid level yielded no significant increase in infectivity to the blood flagellates.

b) Reasoning that in the tsetse fly the developing trypanosomes may well encounter a relatively low oxygen tension in the fly's gut, but during the migration to the salivary glands probably encounter a higher pO_2, we incubated a series of *T. brucei* cultures for ten days with glutathione to reduce pO_2 and then gassed the cultures with oxygen during the remaining eight days prior to injection into mice. From these preliminary experiments no beneficial effect on infectivity could be shown.

c) Because in our cultures the development of infective trypanosomes of *T. brucei* takes about 17 to 18 days, which roughly approximates the time given for the development of metacyclic trypanosomes in the tsetse fly (WENYON, 1926), we reasoned that perhaps a slight diurnal-nocturnal temperature rhythm as encountered in the field might have some influence on trypanosome development. We therefore placed our blood cultures alternately into an incubator at

24°C during the days, and into another at 14°C during the nights, until the flagellates were inoculated into mice on the 17th or 18th day. No apparent influence of this temperature regimen could be detected from these experiments.

By way of summary it may be stated, therefore, that blood from a variety of mammals is capable of producing a certain low percentage of infective *T. brucei* in culture; blood from bovids appears to be the best suited for this purpose, and the blood characteristics of the individual donor is of prime importance. While our investigations seem to show some evidence that non-specific antibodies in the blood of unsuitable donor animals may perhaps play an inhibitory role against the development of metacyclic trypanosomes, these may not be the sole responsible agents and a further investigation of this problem needs to be undertaken. We are at present directing our efforts along these lines.

Acknowledgement

It is a distinct pleasure and honor to acknowledge the wholehearted collaboration of Professor R. Geigy, director of the Swiss Tropical Institute, Basel, in these investigations.

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