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Autor: Steiger, R.F. / Black, C.D.V.
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International Institute of Cellular and Molecular Pathology, Bruxelles, Belgique

Simplified defined media for cultivating *Leishmania donovani* promastigotes*

Short communication

R. F. Steiger, C. D. V. Black

The cultivation of *Leishmania donovani* promastigotes in defined media of the RE series has been described previously (Steiger and Steiger, 1976; 1977). However, these formulas are too complex to elaborate the absolute nutritional requirements of the parasite, inasmuch as they do not represent "minimal essential" mixtures. We were therefore interested in devising simpler media to elucidate more precisely the essentiality of particular nutrients for parasite growth.

Promastigotes of *L. donovani*, Sudan strain 1S (Dwyer, 1977), continuously propagated for more than 50 passages in the RE III medium (Steiger and Steiger, 1977) were successfully adapted by direct transfer to 1) a protein-free (RE IX) and 2) a protein- and glucose-free modification (RE X) of the RE series (Evans, 1978), the compositions and preparation of which are summarized in Table 1.

The parasites were kept at 27–28° C in a final volume of 5 ml of medium in T-25 Falcon tissue culture flasks and serially subcultured once a week and adjusted to an initial density of 10^6 parasites/ml. To date, these cells have been maintained in the new media for more than 30 passages. A total of 200 culture passages of this strain have been effected since its isolation from a hamster (Keithly, 1976). Growth parameters were the same as described elsewhere (Steiger and Steiger, 1977). Maximal cell yields in early stationary phase, 3.5×10^7/ml, in RE IX and RE X are comparable to those achieved in the more complex RE media (Steiger and Steiger, 1976; 1977), with respective population doubling times of 19.2 ± 2.0 (S.D.) and 24.6 ± 1.1 (S.D.) hours for the RE IX and RE X version.

These promastigotes (5×10^7 parasites) proved infective to hamsters upon intraperitoneal and intrasplenic inoculation.

* Dedicated to Prof. W. Trager on the occasion of his 70th birthday

Correspondence: Dr. R. F. Steiger, ICP, Avenue Hippocrate 74, B-1200 Bruxelles, Belgique

Table 1. Composition and preparation of the media RE IX and RE X

Components per liter:

A)	8.0 g	NaCl	C)	300 mg	L-glutamine
	400 mg	KCl		1.0 g	$NaHCO_3$
	200 mg	$MgSO_4.7H_2O$		14.25 g	HEPES (= 60 mM)
	60 mg	$Na_2HPO_4.2H_2O$		20 mg	adenosine
	60 mg	KH_2PO_4			
	2.0 g	glucose – omitted from RE X	D)	1 mg	D-biotin
				1 mg	choline chloride
				1 mg	folic acid
B)	100 mg	L-histidine		2 mg	i-inositol
	100 mg	L-isoleucine		1 mg	niacinamide
	300 mg	L-leucine		1 mg	D-pantothenic acid (hemi-calcium salt)
	250 mg	L-lysine.HCl			
	50 mg	L-methionine		1 mg	pyridoxal.HCl
	100 mg	L-phenylalanine		0.1 mg	riboflavine
	300 mg	L-proline		1 mg	thiamine.HCl
	400 mg	L-threonine			
	50 mg	L-tryptophan	E)	2.5 mg	haemin
	50 mg	L-tyrosine			
	100 mg	L-valine	Redist. H_2O Q.S. 1 liter		

A, B, D, from frozen stock solutions: 2×, 5× and 100× (BME Vitamins, GIBCO), respectively. C, weighed in directly. E, from a filter-sterilized 200× stock prepared in 1 N NaOH.
The pH of the medium is adjusted with 1 N KOH to 7.3 and the medium sterilized with a Millipore filter 0.22 μm. The haemin solution is added at the end. Aliquots of the complete medium (5 ml) are then dispensed sterily into Falcon flasks and stored frozen at –20° C until use.
Note that the media are free of a pH indicator (Phenol red) and antibiotics.

As compared to the more complex RE III medium (Steiger and Steiger, 1977) the new formulas contain lower concentrations of folic acid and haemin, lacking bovine albumin and lipoic acid altogether. In contradiction, glucose was found to be non-essential though growth accelerating. When adenine was substituted for adenosine, the cells failed to grow in the glucose-free version, but not in the glucose-containing RE IX mixture. This might indicate that the ribose moiety is essential for growth when glucose is absent and that promastigotes seem to lack a substantial gluconeogenic activity (Mukkada, 1977), whereas the pentose phosphate shunt is operating when glucose is provided (Martin et al., 1976). It is unclear, however, whether ribose at the concentration tested (0.075 mM) can fulfill all the carbohydrate requirements or if it merely contributes to nucleotide formation in the purine salvage pathway (Marr et al., 1978). At least marginal glucogenic activity could then not be excluded.

The fact that albumin was not required although somewhat beneficial for growth (Steiger and Steiger, 1977), probably makes *L. donovani* different from *Trypanosoma cruzi*, in which haemoproteins (catalase, peroxidase, haemoglobin) are not only growth-promoting but amply meet the nutritional amino acid

requirements of the parasite (Avila et al., 1979). In contrast to the latter organism, a defined balanced amino acid supplement can substitute for a growth-promoting protein in *L. donovani.*

Based on the above and previous data on the amino acid utilization (Steiger and Meshnick, 1977), it must be assumed that proline is the major energy substrate of *L. donovani* (Krassner and Flory, 1972) and that several other amino acids, such as arginine, asparagine and glutamine, may serve as glucogenic substrates. It is obvious that promastigotes of *L. donovani* do not have a specific lipid requirement at lower temperatures and that they possess *de novo* synthetic and desaturase pathways (reviewed by Beach et al., 1979). It can further be hypothesized that, as in *T. brucei* (Linstead et al., 1977), acetate precursors for fatty acid biosynthesis are produced by threonine cleavage.

The absolute requirements for vitamins and related growth factors are far from clear. Analogous needs of *L. donovani* can be inferred from the established requirements of other trypanosomatids (Trager, 1957; 1974), but it still remains unclear if exogenous choline and/or inositol are essential for phospholipid biosynthesis. Such probable requirements might provide useful chemotherapeutic leads reminiscent of anticoccidial agents acting as thiamine analogues (Ryley and Betts, 1973).

Therefore, the types of media described here are invaluable for exploring such prospects. Moreover, protein-free fully defined formulas may prove essential for the production of parasite antigens devoid of serum contaminants, such as "excretion factors" (Decker and Janovy, 1974) and surface membranes.

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