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RAPID COMMUNICATION

A NEW METABOLIC LABELLING MEDIUM FOR TRICHOMONAS VAGINALIS AND TRITRICHOMONAS FOETUS USING ^{35}S METHIONINE

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Abstract. A metabolic labelling medium was devised for Trichomonas vaginalis and Tritrichomonas foetus utilizing ^{35}S methionine. T. vaginalis cultured for 24h in the medium took up approximately 27% of the available label and increased greater than two fold in number. Counts per microgram of protein were 32,555 ± 10% between different strains or identical strains in different labelling runs. T. foetus took up approximately 5% of the available label and increased greater than two fold in 24h. This resulted in specific labelling of 12,704 cpm/ug protein ± 10% between different runs with the same strain. © 1986 Society for Experimental Biology and Medicine

INTRODUCTION

Although axenic culture media have been devised for important parasitic protozoa such as Giardia lamblia, Trichomonas vaginalis and Entamoeba histolytica, successful media contain undefined components such as peptones and serum (1). Intrinsic labelling of these protozoa with amino acids is conceptually difficult because of the large amounts of free amino acids or small molecular weight peptides in these undefined media. Two approaches to radiolabelling can be taken: 1) the use of large amounts of label resulting in poor labelling or 2) the use of a medium which although defined, permits short-term survival but not growth. Intrinsic labelling is not a problem unique to protozoa, but is a problem for mycoplasmas and other organisms for which defined media have yet to be devised. However, protozoa can pinocytize and utilize large molecules for cellular maintenance and growth, and T. vaginalis can ingest organisms such as mycoplasmas, chlamydiae, and bacteria (2) presumably for nutrients. In order to limit competition of small molecular weight medium components with label, we dialyzed both the serum and the peptone/yeast components of a modified Diamond's TYI-S-33 medium (1) and used the non-dialyzable fraction as the base for a labelling medium. Both T. vaginalis and T. foetus showed limited multiplication in this medium and demonstrated good radiolabel uptake and incorporation into protozoal proteins.

MATERIALS AND METHODS

Organisms and Culture. T. vaginalis and T. foetus were cultured in Diamond's TYI-S-33 medium modified by substitution of Biosate (BBL, Cockysville, MD) for casein hydrolysate and yeast extract. Organisms were cultured, tested for contamination with mycoplasma (3), harvested as previously described (4), washed three times in sterile phosphate buffered saline (PBS, 0.01 M phosphate, 0.15 M NaCl, pH 7.0), and the final pellet was resuspended in 2.5 ml of sterile PBS, pH 7.0. Cell

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numbers were assessed by removing 100 ul of the cell suspension, fixing in formalin (1% in PBS), and counting with a hemacytometer. For *T. vaginalis* and *T. foetus*, 2×10^7 cells were used for each radiolabelling.

Dialyzed Biosate. Eighty grams of dry Biosate was poured into a previously boiled, 115 cm (knot to knot) wet piece of dialysis tubing (Spectrapor, molecular weight cut off 12,000 to 14,000, dry diameter 27 mm, dry thickness 0.0008"; Spectrum Medical Industries Inc., Los Angeles, CA) using a funnel and 20 cm piece of Tygon tubing (inner diameter 5/8", Nalgene). The material was dialyzed for 96 h at 4° C against four changes of distilled water (20 liters per 24 hours) in a 20 liter carboy. After 96 h, the material remaining in the dialysis tubing was removed (approximately 1050 to 1100 mls) and a 100 ml aliquot was lyophilized and weighed. Dialyzed Biosate contained 7.15% of the original dry weight of 80 grams.

Serum. One liter of newborn calf serum was dialyzed against distilled water (three changes of 20 liters each) for 96 hours. The volume of retentate in the dialysis tubing was then measured and subsequently made 0.15 M in NaCl, sterilized by filtration through a 0.22 nm filter (Millipore, Bedford, MA), aliquoted, and frozen at -20°C until use.

Complete Dialyzed Medium. Dialyzed biosate (870 mls) was placed in a 1 liter Erlenmeyer flask. With stirring, 10 grams dextrose, 1 gram cysteine hydrochloride, 1 gram K_2HPO_4, 0.6 gram KH_2PO_4, 2 grams NaCl, and 22.5 mg ferric ammonium citrate were added. Note that these ingredients are virtually identical to those in Diamond's TYI-S--33 medium (1) except the ascorbic acid is omitted. After all components dissolved, 100 ml of dialyzed serum and 30 ml of Diamond's Tween-80 Vitamin Mixture (K.C. Biologicals, Lenexa, KS) were added. The medium was brought to pH 6.7 by the addition of 10 N sodium hydroxide, sterilized by filtration through a 0.22 nm filter, aliquoted in 100 ml portions, and frozen at -20°C for up to 60 days before use.

Labelling of Organisms. Organisms (2×10^7 suspended in no more than 2.5 ml PBS) were placed into 140 ml glass bottles containing 120 ml of complete medium (bottles were 85% full) at 37°C. Immediately, ^{35}S-methionine (250 uCi, specific activity 1086 Ci/mmol, New England Nuclear, Boston, Mass.) was added and the culture was incubated at 37 °C for 24 h. Organisms were harvested by centrifugation at 500 x g for 10 min and washed 3x in 10 ml of sterile PBS. Spent medium and wash material were combined and saved for radioactive counting. The final pellet was resuspended in 10 ml PBS, pH 7.0. One ml aliquots were placed in 1.5 ml microfuge tubes and centrifuged at 12,500 x g for 2 min. Supernates were removed by aspiration with a Pasteur pipette and added to the spent medium and wash material. Pelleted cells were frozen at -70°C until use. Protein content was estimated by the method of Bradford (5).

Radioactive Counting. Samples of washed cells (5 or 10 ul in PBS) or samples of spent culture medium (5 ul) were mixed in 10 ml of Aquasol (New England Nuclear, Boston, MA). This was shaken vigorously and counted for 1 min on a Beckman LS 3800 Scintillation Counter.

SDS-PAGE and Fluorography. SDS--PAGE was carried out by a modification of the method of Laemmli (6) as previously described (4). Gels were fixed in a solution of 30% methanol, 10% acetic acid, and 10% trichloroacetic acid for 1 h, treated for 30 min with Enhance (New England Nuclear, Boston, Mass.), then 30 min with distilled water and dried for 1 h at 80°C under vacuum. Fluorography was performed by exposing gels for 3 to 5 days (72 to 120 h) to Kodak XRP-1 film at -70°C.

RESULTS

Radiolabelling and Growth of Organisms in Dialyzed Medium. In previous attempts to label *T. vaginalis* and *T. foetus*, we employed a number of reported strategies with poor results: counts were low per ug of protein. In the dialyzed medium, both *T. vaginalis* and *T. foetus* showed excellent viability (greater than 99% by vigorous mo-

TABLE 1

Radiolabel Accountability

	T. vaginalis	T. foetus
Cell Input	2.0×10^7	2.0×10^7
Cells Harvested (24 h)	4.42×10^7	4.94×10^7
CPM Input (theoretical)	4.95×10^8* (0.25 mCi)*	4.95×10^8* (0.25 mCi)*
CPM Recovered in Cell Pellet (3x washed)	1.31×10^8	2.54×10^7
CPM Recovered in Cell Supernatant & Washes	3.43×10^8	4.61×10^8
Total CPM Recovered	4.74×10^8	4.86×10^8
% Recoverable Label Incorporated into Cells	27.6%	5.22%
% Theoretical Available Label Incorporated into Cells	26.4%	5.13%
Total % Theoretical Label Recovered	95.7%	98.2%
CPM/ug Protein	32,555	12,704

* Based on 90% counting efficiency

tility) after incubation for 24 h. More than a two-fold increase in number of organisms was observed (Table 1). The uptake of ^{35}S-methionine into the cells was 27.6% for T. vaginalis and 5.2% for T. foetus. Incorporation of label per ug of cell protein was high: 32,555 cpm per ug for T. vaginalis and 12,704 cpm per ug for T. foetus. When higher concentrations (2×10^8) of organisms were used, more radiolabel was taken up (up to 40% for T. vaginalis) but the specific activity per ug protein was significantly lower (data not shown). Furthermore, at these greater cell concentrations, the cultures had to be split 1:2 in the labelling medium (without the addition of more label) in order to maintain viability. The labelling procedure for T. vaginalis was repeated twice with the CDC strain and four times each with strain PHS-2J and STD-24. Total label incorporation ranged from 1.19×10^8 to 1.42×10^8 cpm. Percent incorporation did not vary more than 10% between experiments when different strains were used at equivalent starting densities.

Fluorography. The labelling intensities for T. vaginalis and T. foetus were such that a three day fluorograph of 10 ug protein resulted in the visualization of at least 20 discrete radiolabelled components (Fig. 1).

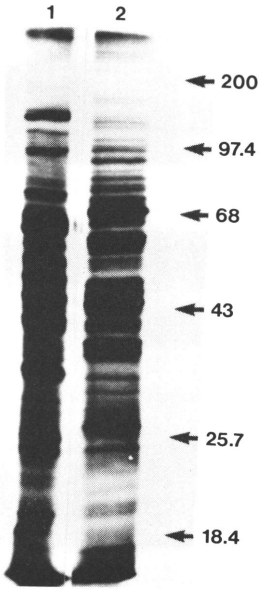

Figure 1. Profiles of T. foetus and T. vaginalis labelled with ^{35}S-methionine run in 10% SDS-PAGE gels. Fluorograph of 1) T. vaginalis (10 ug protein; 300,000 cpm) and 2) T. foetus (10 ug protein; 125,000 cpm) after 96 h exposure.

DISCUSSION

Both T. vaginalis and T. foetus can survive and show limited replication in a complex medium which has its small molecular weight fraction removed by dialysis. Since the organisms do not show similar survival in saline solutions or even in tissue culture media supplemented with serum, they apparently can obtain their nutrients by hydrolysis of large molecular weight components in the medium (the retentate and the dialyzed serum component). The results appear to indicate that these protozoa do not require small molecular weight components (less than 14,000 MW) for the limited growth shown in these experiments. As a consequence, the dialyzed medium provided for excellent ^{35}S-methionine labelling of both species since competition by small molecular weight constituents had been eliminated. Approximately 27% of the label was incorporated by T. vaginalis and 5% by T. foetus. Although different strains of T. vaginalis took up similar amounts of radiolabel, uptake by T. foetus was much less for unknown reasons. The result is an easy, reproducible, and cost-effective method for metabolically labelling T. vaginalis and T. foetus. Although we have not extended the scope of usefulness beyond these two parasites, it is likely that other fastidious organisms such as E. histolytica and G. lamblia could survive and be efficiently labelled using this medium because the parent medium (Diamond's TYI-S-33) was devised for the culture of amoebae and gives excellent culture results with G. lamblia. Under ideal conditions, radiolabelling efficiency with methionine (cpm/ug protein) depended largely on the number of organisms present, whether the organisms were dividing, the amount of label added, and the time allowed for label incorporation. Twenty-four hours produced efficient labelling when 2.0×10^7 organisms were used as inoculum (1.5×10^7 per ml). However, large increases in organism input resulted in loss of viability between 12 and 24 h of incubation. This observation is not surprising, since the retentate base contained only 7.15% (w/w) of the original Biosate material and the medium contained dialyzed serum. It is also likely that labelling efficiency with respect to cpm incorporated would be significantly greater if two to three times more label were added to the cultures. Such an increase might be desirable, especially in cases where a polypeptide of interest was not quantitatively well-represented in the cells. Alternatively amino-acid mixtures could be used to label T. vaginalis or other protozoa in this medium, thus labelling polypeptides deficient or lacking in methionine. The concept of dialyzing a complex medium to eliminate small molecules which compete with radiolabelled amino acid incorporation into protozoal proteins should prove useful in a number of parasite culture systems.

ACKNOWLEDGEMENT

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