*Mem. Inst. Oswaldo Cruz*, Rio de Janeiro
Vol. 83(2): 265-266, abr./jun. 1988

RESEARCH NOTE

# CONTROL OF MYCOPLASMA CONTAMINATION IN HYBRIDOMA TECHNOLOGY

VERA BONGERTZ* & NEIDE THOMAZ**

Instituto Oswaldo Cruz, * Departamento de Imunologia ** Departamento de Protozoologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

We would like to report our recent experience with control of Mycoplasma contamination in tissue cultures. Work with cell cultures in the tropics is often difficult, as contaminations – probably due more to the high air humidity than to the hot weather – occur much more frequently than in more temperate regions.

In our laboratory, work on cell cultures goes on in parallel to work with live animals. As we have a problem with Mycoplasma contamination of our animal stocks, we have to consider this problem when cell cultures are made from animals originated from our breeding facilities. Mycoplasma contamination has been described as being responsible for a wide range of biological, biochemical and immunological artifacts (E. J. Stanbridge, 1981, *Isr. J. Med. Sci., 17:* 563).

Mainly affected by Mycoplasma contamination are long-term cultures, such as those produced using the hybridoma technology. We knew until recently only of Tylan$^R$ (Elanco/Ely Lily) as Mycoplasma-static drug. While using this drug successfully for cell cultures, we have noticed that replication rates are significantly lower than with non-contaminated cells (Fig.); without Tylan$^R$, however, we have no replication at all. Now we have heard of "TRICINE" [N-tris (hydroxymethyl) methylglycine], a buffer developed for use in cell cultures in substitution of HEPES. This substance, as shown by Spendlove et al. in 1971 (*Proc. Soc. Exptl. Med. Parasitol., 137:* 258) inhibits Mycoplasma growth in cell cultures, in addition to its buffering properties. Tricine has not found as great an acceptance by cell culture workers as should be expected, possibly due to interference with cell attachment to culture vessels (Good et al., 1966, *Biochem., 5:* 467). However, the hybridoma technology does not depend on attachment of cells to culture vessels, and therefore the use of Tricine is indicated.

We have tested Tricine (Merck 8602), at the concentrations indicated by Spendlove et al., both in cloning through limiting dilution and in batch cultures.

Four different culture media were tested. To our basic medium (RPMI 1640 + 10 mM Glutamine + 100 U/ml Penicillin + 10% (batch cultures) or 20% (clonings) fetal calf serum, the substances described below were added:

(1) 5 mM sodium bicarbonate, 10 mM HEPES*
(2) 5 mM sodium bicarbonate, 10 mM HEPES, 0,002% Tylan$^R$
(3) 5 mM sodium bicarbonate, 10 mM Tricine
(4) 23 mM tricine

Cloning was carried out through limiting dilution in 96 well microtiter plates. Feeder layers (mouse peritoneal macrophages) were prepared in basic medium ($10^4$ cells/well), allowed to adhere, the medium taken off and suspensions of one of our Balb/c-derived hybrids in the 4 different media were added, in a dilution correspondent to 0.5 cells/well. After 7 days, growth was observed in all plates but that containing cells resuspended in 23 mM Tricine (medium 4). While the plate containing cells in HEPES medium (1) showed growth of 5 hybrids after 7 days, none of these survived more than 14 days. The plate containing Tylan$^R$ (medium 2) showed growth in 5 wells, but only 3 of the clones proliferated; 50% of the well area being filled up by day 23. In contrast, all 20 clones observed in the plate containing 10 mM Tricine (medium 3) proliferated extensively, so that 50% of the growth area was filled up between days 12 and 14. Antibody production was monitored using an ELISA test, and indicated that neither Tylan$^R$

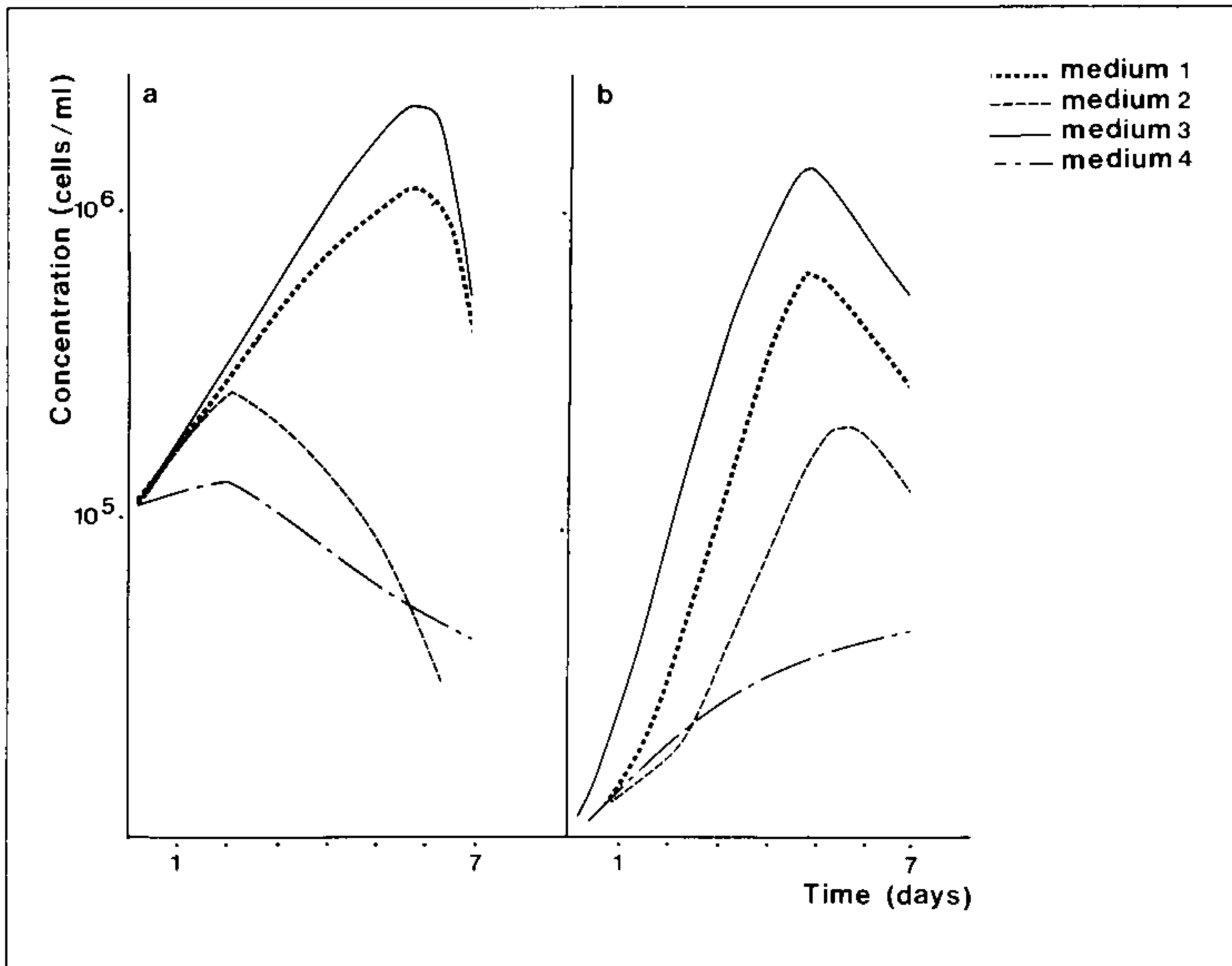
Received February 11, 1988.
Accepted March 24, 1988.

* HEPES = (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

nor Tricine interfered with antibody secretion or activity.

In parallel to the cloning experiments, the Sp2/0 myeloma cell and one of our Balb/c-derived clones were grown in batch cultures in the different culture media described above. Cells were being passaged in medium with Tylan$^R$ (medium 2) before analysis of growth in the different culture media. Cell growth was monitored during 7 consecutive days (see Fig.). Thereafter, the cells were resuspended in fresh culture medium ($10^6$/ml) and passaged upon confluency. Cells maintained in medium containing Tylan$^R$ were passaged every 8 days, indefinitely. The medium (4), containing the higher concentration of Tricine, did not allow sufficient cell growth for attainment of confluency, and was therefore discarded. Medium containing no Mycoplasma-static substance (medium 1) showed good growth during the first passage (see Fig.), but thereafter growth rates slowed down significantly, and the cells had to be discarded. In contrast, Tricine at 10 mM (medium 3) allowed cell growth at the rates described for non-contaminated cells (passages every 3 days) for more than 30 consecutive passages made up to now.

We conclude that Mycoplasma contamination of hybrids can be controlled by substitution of HEPES buffer with Tricine, at a concentration of 10 mM, without affecting neither growth rate nor antibody secretion or activity.




Growth of Myeloma cells and of hybrids in batch cultures. All cells were pretreated with Tylan$^R$ during 2 sequential passages. (A) Sp2/0 myeloma cell, (B) M3B4, an anti-*Trypanosoma cruzi* monoclonal Balb/c-derived hybrid.