FOLIA PARASITOLOGICA 39: 285–286, 1992.

IN VITRO PROPAGATION OF *TOXOPLASMA GONDII* IN PC-1 SERUM-FREE MEDIUM

So far, *in vitro* cultivation of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) has always been performed in serum or yeast-extract enriched media, thereby introducing an uncontrolled set of proteins into the parasites environment. Moreover, serum containing propagation techniques always include the risk of introducing (diaplacentarily transmitted) anti-*Toxoplasma* antibodies. Until now, one notice has only been published describing the cultivation of *T. gondii* in host cells raised in serum-free media (SHITE and Iscove's medium) (Hermentin et al. 1987: J. Parasitol. 73: 1276–1277). However, these media had to be enriched with growth-stimulating supplements, making serum-free *Toxoplasma* cultivation complicated and expensive. Thus, we searched for an easy-to-use serum-free nutrition medium which would enable the simultaneous multiplication of both host tissues and *Toxoplasma* parasites. We cultivated the parasites (strain BK, virulent) in HEp-2 host cells (CCI 23 ATCC; 2×10^6 tachyzoites to 5×10^5 host cells in 25 cm^2 flasks) raised in the ready-to-use, serum-free and low-protein medium Ventrex PC-1 (Ventrex Laboratories Inc; Portland; ME) on one hand (sf culture technique) and in Earl's medium with 10 % heat inactivated fetal calf serum on the other (conventional (conv.) culture technique) according to the technique described by Hermentin et al. (1987: Zbl. Bakt. Hyg. A 267: 272–276). All cultures were harvested on day 4 p.i.; all data of three consecutive experiments were evaluated. For comparison, *T. gondii* trophozoites were also routineously maintained in the usual way by continuous intraperitoneal passages in SPF-mice (OF 1-Swiss, n = 20, three experiments) (43 h cycle).

On day 4 p.i. about 8×10^7 tachyzoites were harvested in both tissue cultures, the *Toxoplasma* trophozoites had multiplied about 40-fold (conv: x = 42, SD 2.5; sf: x = 40, SD 1.76; statistically NS, *t*-test). The multiplication rate of the parasites in the mouse peritoneal cavity was about 15-fold within 43 hours. Although there is almost the same quantity of parasites maturing in both types of tissue cultures, there is a statistically highly significant ($n > 90$; $p < 0.0005$; Welch-test) difference in the parasite size (length: conv: x = 4.01 μm, SD 0.44; sf: x = 5.74 μm, SD 1.0; mouse: x = 5.0 μm, SD 0.71); size measurement done with an ocular micrometer. The trophozoites increases in the average size by about 40 % in PC-1 medium, they even become more voluminous than those grown in the mouse peritoneal cavity. It seems that the parasite "feels quite comfortable" during its multiplication in the PC-1 medium poor of any inhibiting factors.

As *Toxoplasma* trophozoites seem to adsorb some environmental proteins, especially antibodies, to their surface (e.g. Budzko et al. 1989: J. Clin. Microbiol. 27: 959–961) the antigen composition of the surface may change considerably during any serum-free propagation. Thus, we compared the major proteins of *T. gondii* in a reducing SDS-PAGE (2×10^8/ml trophozoites, gradient gel 10–15, PhastSystem; Pharmacia LKB Biotechnology; Vienna; staining with Coomassie blue; all done according to the manufacturer's recommendations). As can be seen in the image (Fig. 1) from the gel, scanned in a PhastImage and redrawn with a graphic software, protein composition of the parasites grown under different environmental conditions do not change considerably, except in the low molecular weight area (mw < 30 kD). Yet, this discoincidence might be due to differences in the protein shattering during preparation. Noteworthy, the peak with a molecular weight of about 30 kD in conventional tissue culture shifts; just this protein is a very prominent *Toxoplasma* surface antigen (e.g., Kasper et al. 1983: J. Immunol. 130: 2407–2412). Thus, before using this *in vitro* propagation technique for an antigen production for serotests the diagnostic relevance of such serological assays has to be verified.

So far, this is the first report of a propagation of *T. gondii* in Ventrex PC-1 medium, a serum-free, low-protein tissue culture medium, which is – according to the producer – a modified DME:F12 base with less than 530 μg proteins/ml (further details are not available). No adaption either of

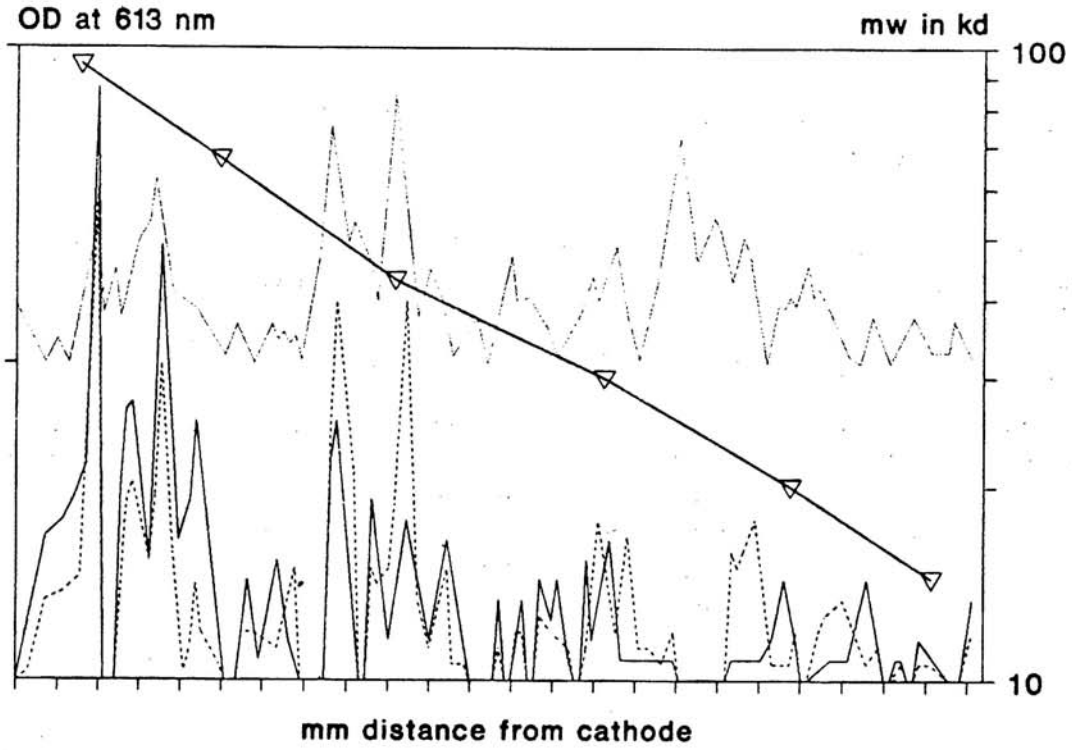

Fig. 1. Graph of a dyed and scanned polyacrylamid-gel after a SDS-electrophoreses under reducing conditions. The samples were lytic proteins of *Toxoplasma gondii* trophozoites grown in mouse peritoneal cavity (dotted line), in a serum enriched HEp-2 tissue culture (dashed line), or in a PC-1 serum-free HEp-2 tissue culture (unbroken line). The dotted line was 50% upset and lifted. From both graphs of tissue culture grown *Toxoplasma* (dashed and unbroken line) all peaks were eliminated which were identical with peaks prepared from uninfected HEp-2 cells. The triangles mark the sites of commercially available molecular weight markers (from left: 94, 67, 43, 30, 20.1, and 14.4 kD).

the HEp-2 cells or of the parasites to the serum-free environment by a gradual decrease of serum concentration in the nutrition medium has been necessary for their propagation. Thus, a very convenient and reproducible technique for an *in vitro* multiplication of *T. gondii* is offered, which produces rich yields of remarkably large parasites.

This study has in part been supported by the grant P7151-MED of the Austrian Fonds zur Förderung der wissenschaftlichen Forschung.

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Received 30 December 1991

Accepted 2 March 1992