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Induction of differentiation in *Herpetomonas samuelpessoai* by dimethylsulfoxide

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Summary

Dimethylsulfoxide (DMSO) induces in *Herpetomonas samuelpessoai*, grown in a chemically defined medium at 28° C the transformation of promastigotes into paramastigotes and opisthomastigotes. This effect was dependent on the period of cultivation and the concentration of DMSO. Initially, DMSO induced the appearance of paramastigotes and later, of opisthomastigotes. Approximately 43% opisthomastigotes were obtained after growth of the protozoan for 96 h at 28° C in a medium containing 3% DMSO. No ultrastructural changes were observed on DMSO-treated cells. Based on these results and others previously described, the process of differentiation in *Herpetomonas* is compared and analyzed with that occurring in *Trypanosoma.*

Key words: Herpetomonas samuelpessoai; Trypanosomatidae; Protozoa; cell differentiation; dimethylsulfoxide.

Introduction

Some genera of the Trypanosomatidae family are able to undergo a process of differentiation in which one developmental stage transforms into another. The systems which have been studied in some detail up to now are (a) the amastigote-promastigote transformation found in *Leishmania* (Rudzinska et al., 1964; Simpson, 1968; Dwyer et al., 1974; Brun and Krassner, 1976), (b) the epimastigote-trypomastigote transformation found in *Trypanosoma cruzi* (Camargo, 1964; Brener, 1972; Astolfi et al., 1978; Lanar, 1979), (c) the bloodstream trypomastigote-procyclic trypomastigote transformation found in *Try-*

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panosoma brucei (Brown et al., 1973; Ghiotto et al., 1979) and the promastigote-opisthomastigote transformation found in *Herpetomonas* (Janovy et al., 1974; Roitman et al., 1976; Knight, 1976; Angluster et al., 1977; De Souza et al., in press; Thomas et al., in press).

In relation to *Herpetomonas* few attempts have been made in order to induce cell differentiation (Roitman et al., 1976; Knight, 1976; Angluster et al., 1977; De Souza et al., in press; Thomas et al., in press). Assuming that the process of cell differentiation may result from changes in the plasma membrane of the protozoan, we have tried to induce the promastigote-opisthomastigote transformation in *Herpetomonas samuelpessoai.* We have found that the plant lectin, Concanavalin A (De Souza et al., in press), and the anesthetic, lidocaine (Thomas et al., in press), which interact with plasma membrane components, trigger the process of cell differentiation in *H. samuelpessoai.*

In the present report we describe the results obtained by using dimethylsulfoxide (DMSO) to induce cell differentiation in *H. samuelpessoai.* This substance has been used in the last years as an inducer of differentiation of Friend leukemia cells along the erythroid pathway (Friend et al., 1971; Ross et al., 1972; Tanaka et al., 1975; Kluge et al., 1976; Lyman et al., 1976; Collins et al., 1977; Newburger et al., 1979). It has also been shown that DMSO alter the properties of membrane phospholipids, as seen by differential scanning calorimetry, causing the appearance of a new transition at higher temperature which leads to a decrease in the membrane fluidity.

Materials and methods

Herpetomonas samuelpessoai (ATCC 30252) was maintained by weekly transfers in a chemically defined medium (Roitman et al., 1972). Experiments were made in 18×150 mm glass tubes containing 5 ml of defined medium. Dimethylsulfoxide (DMSO) was filter-sterilized (Seitz filter) and then added to the culture medium in final concentrations ranging from 0.4 and 3.0%. The inoculum consisted of 0.1 ml of a 48-h culture containing about 4.10^7 cells/ml. Cells were grown at 28° C for periods ranging from 24 to 96 h. Cell growth was estimated either as absorbance at 540 nm or by counting the cells in a Neubauer chamber. After 24, 48, 72 and 96 h of cultivation, the percentage of promastigote, paramastigote and opisthomastigote forms was determined using Giemsa stained preparations. At least 200 organisms were examined in each preparation. In order to find out if DMSO-treated cells were viable, they, as well as non-treated cells, were transferred to fresh media and the cell growth followed. For electron-microscopic examination the cells were processed as previously described (De Souza et al., 1976). All assays were performed in duplicate. Each experiment was repeated at least 3 times.

Results

Herpetomonas samuelpessoai grown in a medium containing less than 0.4% DMSO, multiplied at approximately the same rate as did control cells. With increasing concentration ($\geq$0.4% DMSO) a progressive inhibition of cell growth occurred. However, total inhibition was not observed, even at concentrations

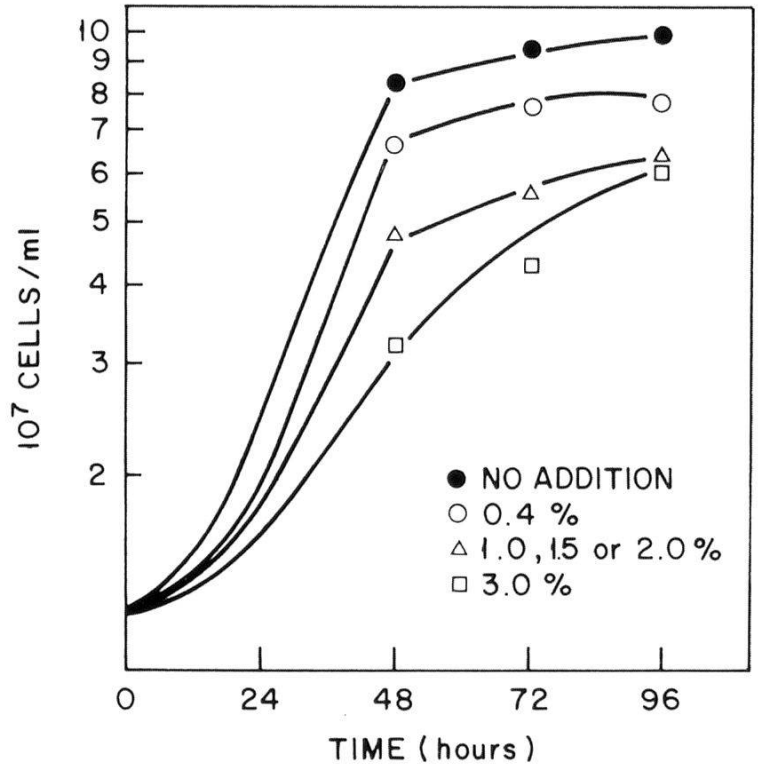

Fig. 1. Effect of dimethylsulfoxide (DMSO) on the cell growth of *Herpetomonas samuelpessoai* in a chemically defined medium at 28° C. Data are from one representative experiment.

of 3% DMSO (Fig. 1). We did not try to use higher concentrations of DMSO since all previous studies on DMSO-induced cell differentiation used concentrations ranges between 1.0 to 3.0%. Higher concentrations are usually toxic for all cells tested, including *H. samuelpessoai* (Friend et al., 1971; Tanaka et al., 1975; Kluge et al., 1976).

Light microscopic observations of the cultures indicated that in all concentrations of DMSO tested, no changes occurred in cell motility. Also, no gross morphological alterations were observed.

Fig. 2 summarizes the results obtained from the examination of the Giemsa-stained preparations for the determination of the percentages of the three developmental forms found in *H. samuelpessoai* cultures: promastigote, paramastigote and opisthomastigote. As previously described (Roitman et al., 1976; Angluster et al., 1977), *H. samuelpessoai* grown in a chemically defined medium at 28° C showed a low rate of differentiation. Few if any opisthomastigotes were found in the first 48 h of cultivation. Later, however, they made up about 5.0% of the cells. Paramastigotes were always observed even in 24-h cultures. During the stationary phase of growth they made up about 40% of the total cells. Promastigotes were always the predominant forms found in control cultures.

When DMSO was added to the cultures there was a considerable increase in the number of paramastigotes and opisthomastigotes. Although the highest number of these two forms were found at higher concentrations of DMSO, a large number of these two forms were also found at lower concentrations of the

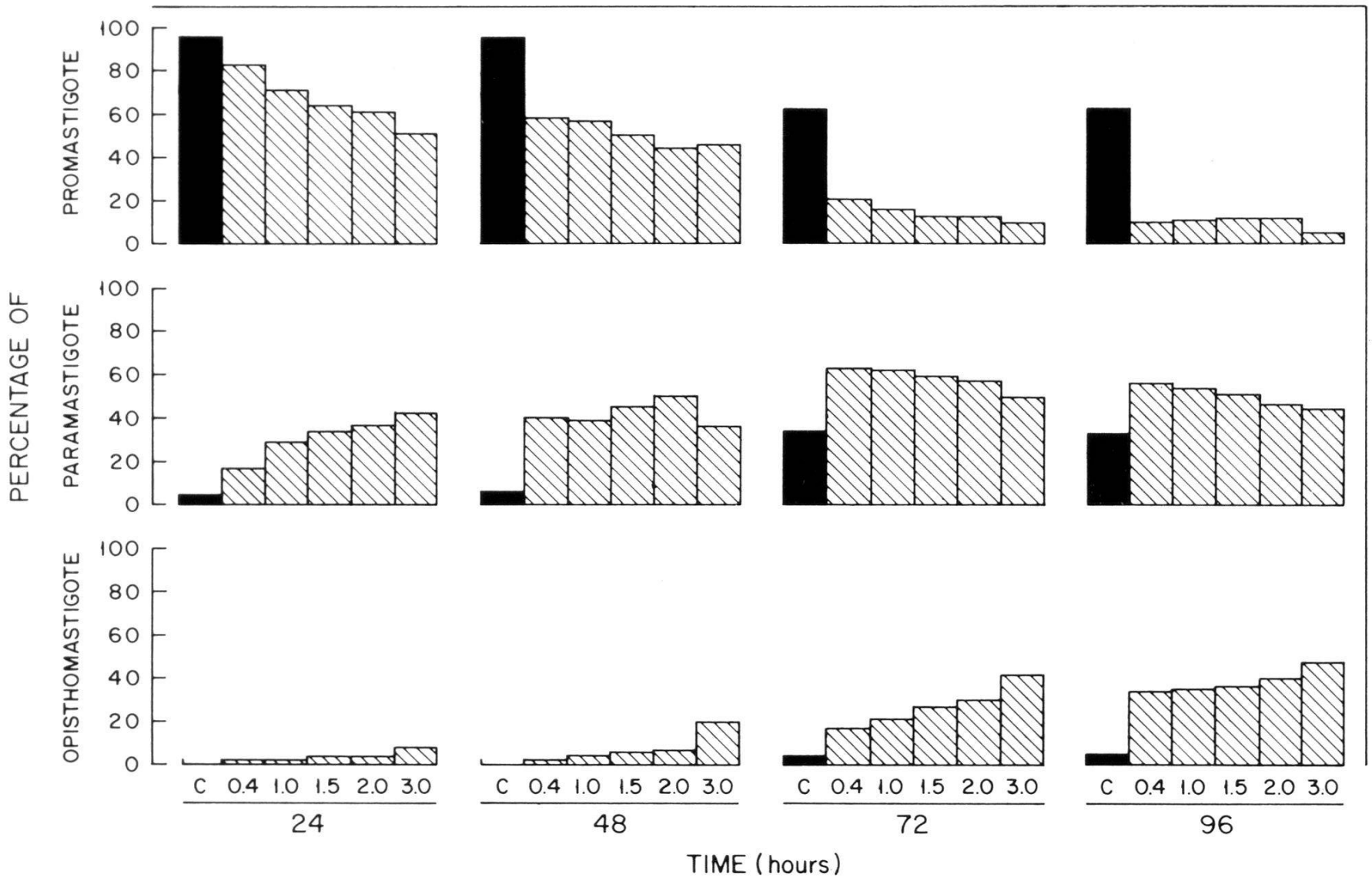

Fig. 2. Effect of dimethylsulfoxide (DMSO) on the differentiation of *Herpetomonas samuelpessoai* grown in a chemically defined medium at 28° C. Data are from one representative experiment. ■ = Control (C); □ = concentrations (%) of DMSO.

drug. The first effect of DMSO was observed in the logarithmic phase of growth as an increase in the number of paramastigotes. In the first 24 h this increase was dependent on the concentration of DMSO. Some opisthomastigotes were also seen in the first 24 h. Their number, however, increased with the time of cultivation, reaching about 40% of the forms found in cultures after 76 h of incubation (Fig. 2). In order to see if such cells were viable, sub-cultures were made. In these experiments samples of 96 h cultures, both from control and DMSO-treated, were inoculated into fresh medium and the cell growth was measured. Both control and DMSO-treated cells showed similar growth-curves.

Most of the opisthomastigotes found in DMSO-treated cells were elongated, as described for *Herpetomonas megaseliae* (Janovy et al., 1974), however, some rounded ones were also seen. By electron microscopy, it was easy to distinguish the three developmental stages of *H. samuelpessoai* as previously described (Roitman et al., 1976; Angluster et al., 1977). We did not observe any important morphological alterations besides the change in the relative position of the kinetoplast to the nucleus.

Discussion

Trypanosomatidae of the genus *Herpetomonas* are usually taxonomically characterized by having the promastigote and the opisthomastigote developmental stages. These two forms are differentiated by the relative position of the kinetoplast to the nucleus. In the two species of *Herpetomonas* which have been examined in more detail, *H. megaseliae* and *H. samuelpessoai* cultures in the logarithmic phase of growth show promastigotes almost exclusively. At the stationary phase of growth, however, some opisthomastigotes have been found. In *Trypanosoma cruzi* epimastigotes are observed in the logarithmic phase of growth. At the stationary phase some epimastigotes start a process of differentiation which leads to the appearance of trypomastigotes. In the culture media usually employed to cultivate *T. cruzi* only a low percentage (5–20%) of the epimastigotes transform into trypomastigotes (Camargo, 1964; Chiari, 1974; Lanar et al., 1979). Analogous to *T. cruzi* we consider that *Herpetomonas* is also able to start a process of cell differentiation, in which the opisthomastigote form would be the more differentiated stage.

During epimastigote-trypomastigote transformation in *T. cruzi* and promastigote-opisthomastigote transformation in *H. samuelpessoai*, there are changes in the spatial organization of the cellular structures, which lead to modification in the relative position of the kinetoplast to the nucleus (Meyer and De Souza, 1976; De Andrade and De Almeida, 1980). In the case of *Herpetomonas* there is a clear intermediate stage which has been designated as the paramastigote (Janovy et al., 1974; Knight, 1976; Roitman et al., 1976; Angluster et al., 1977). Initially we considered this form to be a brief transition stage between promastigotes and opisthomastigotes. However, the recently obtained

results suggest that the paramastigote may represent a well defined stage in the *Herpetomonas* life cycle (De Souza et al., in press; Thomas et al., in press). From the data obtained in the present study (Fig. 2) it seems clear that in DMSO-treated cells there is an initial increase in the percentage of paramastigotes which is subsequently followed by an increase in the number of opisthomastigotes. In experiments using Concanavalin A, we have shown that concentrations of 0.1 to 500 μg/ml of this lectin induce the appearance of paramastigotes (which may be about 70% of the total cells) and completely block the appearance of opisthomastigotes. At lower concentrations, however, Concanavalin A induces the appearance of opisthomastigotes (De Souza et al., in press). These effects of Concanavalin A can be observed at concentrations of the lectin which do not interfere with cell growth.

In the case of *T. cruzi* it is very easy to separate the epimastigote and trypomastigote forms functionally. While the first is not infective for mice and is digested by cultured macrophages, the second is infective both for mice and for macrophage (Nogueira and Cohn, 1976). With respect to *Herpetomonas* we do not have at the present a criterion besides the morphological one, to show that promastigotes, paramastigotes and opisthomastigotes are actually different developmental stages. One approach to this problem would be to obtain large quantities of the three forms, characterize them by morphological, biochemical, and immunological methods, and determine if they are different or not. In order to perform such studies it is first necessary to develop systems in which the three stages can be found.

Several approaches can be used to increase the rate of differentiation of cells. They include (a) changes in the composition of the culture medium, (b) changes in the conditions of cultivation, and (c) the addition to the culture medium of substances which may interact with some components of the cells. The last two approaches have been used to induce cell differentiation in *Herpetomonas.* As shown in Table 1 a higher rate of differentiation has been obtained when *H. samuelpessoai* has been grown (a) in a chemically defined medium at 37° C (Roitman et al., 1976), (b) in the presence of 2-deoxy-D-glucose (Anglister et al., 1977), Concanavalin A (De Souza et al., in press), lidocaine (Thomas et al., in press) and DMSO (present study). In *H. megaseliae* it has been shown that hydroxyurea induces the appearance of opisthomastigotes (Knight, 1976).

There is evidence that drugs which interact with components of the plasma membrane induce dramatic changes in the behaviour of the cells, triggering either the process of cell division, as occurs with lymphocytes (for a review see Nicolson, 1974), or cell differentiation. Dimethylsulfoxide is usually employed as a penetrant-carrier and cryoprotectant. It has been widely used to stimulate the process of cell differentiation of leukemia cells (Friend et al., 1971; Ross et al., 1972; Tanaka et al., 1975; Kluge et al., 1976; Lyman et al., 1976; Collins et al., 1977; Newburger et al., 1979). Its mechanism of action is not yet clear. It has been suggested that it can act either by changing the conformation of DNA or

Table 1. Percentage of opisthomastigote forms found in *Herpetomonas samuelpessoai* grown under different conditions

Conditions*	Percentage	References
Defined medium (28° C)	2.0	Roitman et al., 1976
Complex medium (28° C)	0	
Defined medium (37° C)	34.0	
Complex medium (37° C)	7.0	
Defined medium (28° C) plus 2-deoxy-D-glucose (30.5 mM)	33.7	Angluster et al., 1977
Defined medium (28° C) plus lidocaine (3.0 M)	50.0	Thomas et al., in press
Defined medium (28° C) plus Concanavalin A (0.002 μg/ml)	20.0	De Souza et al., in press
Defined medium (28° C) plus dimethylsulfoxide (3%)	43.0	present study

* The data shown in this table are from the experimental conditions in which the cited authors detected the highest percentages of differentiated forms.

DNA-protein complex causing an alteration in the process of transcription that leads to the expression of genes that regulate cell differentiation (Tanaka et al., 1975), or by inducing changes in the properties of phospholipids, thereby decreasing the fluidity of the plasma membrane (Lyman et al., 1976). Our data show that DMSO also triggers the process of cell differentiation in *H. samuelpessoai.*

The results obtained in the present report and those listed in Table 1, indicate that it is now possible to start studies dealing with the purification and analysis of the three forms found in cultures of *Herpetomonas samuelpessoai.*

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