

Cytoplasmic Changes in the Cell Body of Cystine Treated Amoebae*

By

P. L. D. Waidyasekera

Department of Biological Sciences, Vidyodaya University of Ceylon.

Introduction

A vast amount of literature has accumulated in recent years on the action and effects of chemical substances on the living cell (Lwoff, 1951 ; Zimmerman, 1959). The interest of earlier workers centred mainly on the question of resistance to chemical treatment and survival specially of unicellular organisms as cellular units (Harvey, 1911 ; Gray, 1920 ; Lewis 1923 ; Tunicliff, 1928 ; Phillpot, 1928, 1930 ; etc.). The biochemical mechanisms engaged in the synthesis of amino acids and proteins in the cell have received a good deal of attention (Brachet, 1957). Physiological properties of the cell membrane in relation to the penetration of amino acids and glucose have also been dealt with by various workers (Christenson et al, 1952 ; Danielli, 1952 ; Heinz, 1954 ; Harris, 1956 ; Plaut and Rustard, 1956). In recent years interest has concentrated on the action of various chemicals in relation to the morphogenetic changes in the cell body of amoebae (Hazra 1959 ; 1961 ; Mookerjee & Waidyasekera, 1959 ; Mookerjee & Waidyasekera, 1962 ; Mookerjee & Alamelu, 1959). In some previous communications it has been reported that various concentrations of specific amino acids like Asparagine, Isoleucine and Lysine have different effects on the cytoplasmic and nuclear pattern of trophic amoebae (Waidyasekera, Waidyasekera & Mookerjee, in press). The present work is an extension of this study to the effects of the amino acid, Cystine.

The main object of this paper is to consider some results obtained after exposing trophic amoebae to 0.125% Cystine (E. Merck).

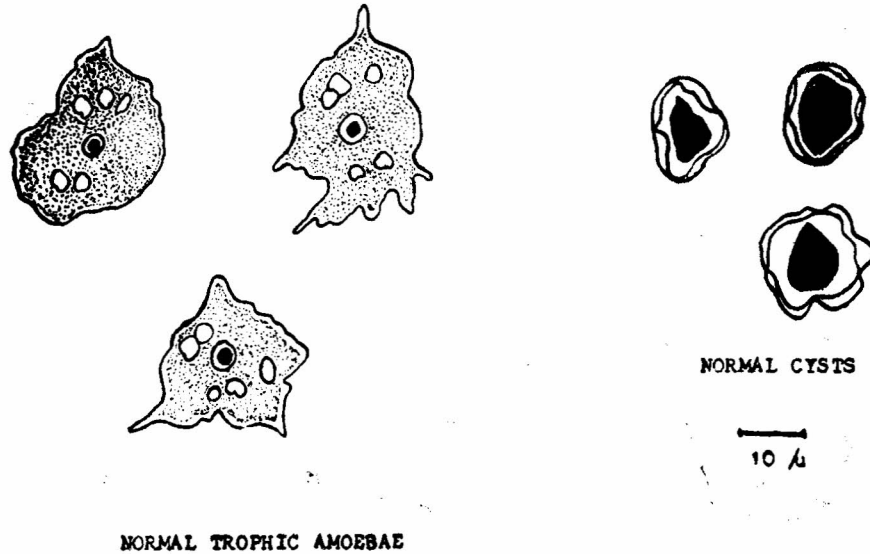
Experimental

Different concentrations of Cystine were made in 0.05% saline (culture solution of amoebae) and 2 to 3 drops of the testing chemical introduced on to slides containing fresh cultures of soil amoebae — *Acanthamoeba* sp. Cultures were done by the method described by Singh (1950) as modified for slides by Mookerjee and Hazra (1960). The amoebae were fed on *Aerobactus* sp. Trophic forms after treatment were subjected to observations at short intervals

*This paper was read at the 22nd annual sessions of the Ceylon Association for the Advancement of Science, December 1966.

until encystment. The cysts were then dried, washed and subcultured. The sequence was repeated to the 4th subculture. The slides were either fixed at regular intervals in Carnoy's fluid and permanent preparations made by the Iron-alum Haematoxylin technique or fixed in Zenker's fluid and subjected to Toludine Blue treatment for basophilic reactions. Among the various dosages used 0.125% was found to be most effective in showing certain changes in the cytoplasm. Slides with normal trophic forms were also fixed as controls.

Cellular configuration of normal amoebae : (Figure 1).



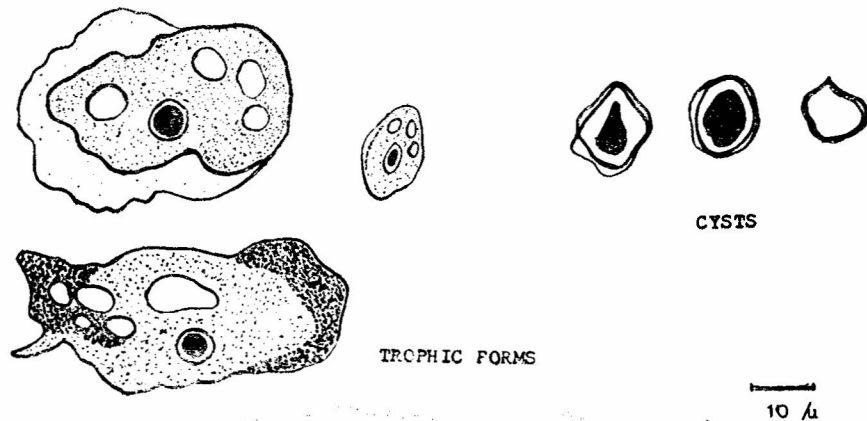
The cytoplasm is evenly stained and the distribution of granules even. Pseudopodia are pointed and needle like. The vacuoles are moderate in size and their number in each amoeba varies from 3-6. Clearly visible ectoplasm and endoplasm are seen. The nucleus remains close to the centre.

CYSTINE TREATED AMOEBÆ

Observations immediately after treatment with 0.125% cystine.

The amoebae remained normal for some time after introduction of the Cystine solution. Then excessive vacuolation started but later the normal structure was regained. The amoebae encysted after 48 hours. The cysts were subjected to a 1st subculture.

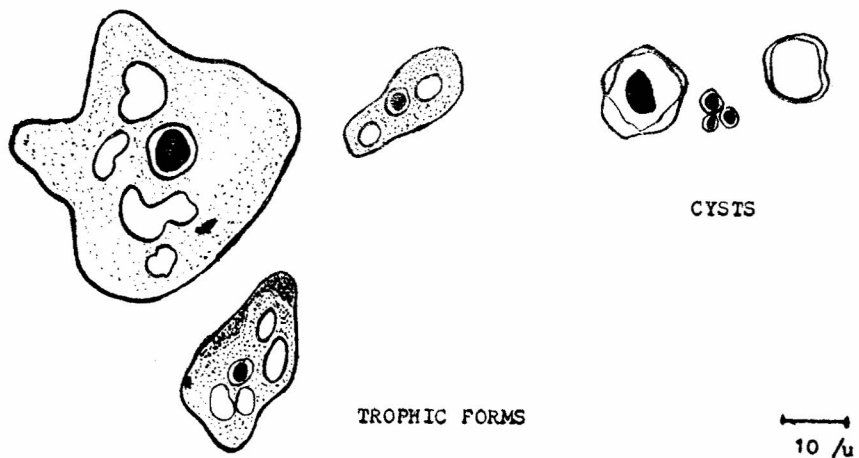
Effects seen in the 1st subculture after treatment : (Figure II).



0.125% CYSTINE- FIRST SUB-CULTURE AFTER TREATMENT

The trophic forms that emerged at the 1st subculture showed two types—large and small amoebae. The stain in the cytoplasm showed darker and lighter patches. Along with this variable nature of the staining ability was seen a peripheral faint band of cytoplasm in certain regions. During 72 hours the dark patches drifted towards the vacuoles. They varied from 4 to 9 in number. Nuclear enlargement was clearly noticed. Trophic forms encysted after 72 hours. The cysts obtained varied from granular types to empty cysts. The toluidine blue reaction was almost the same as the Iron alum Haematoxylin stain.

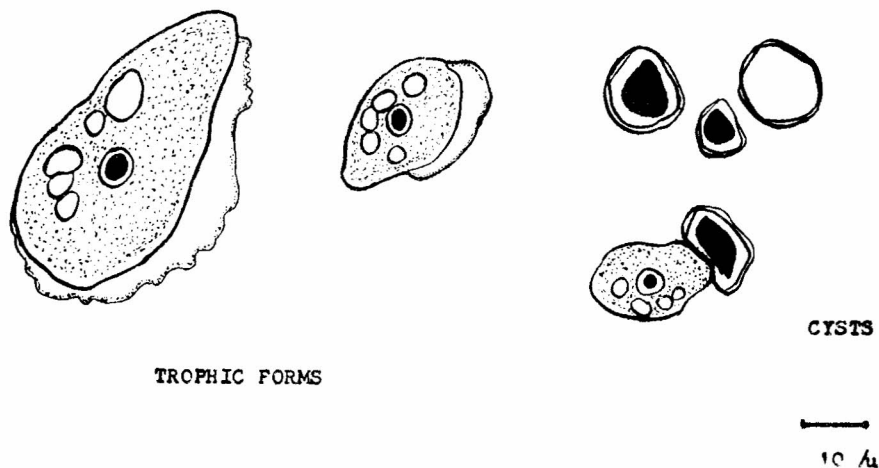
Effects seen in the 2nd subculture after treatment : (Figure III).



0.125% CYSTINE - SECOND SUB-CULTURE AFTER TREATMENT

Cysts obtained from the 1st subculture were subjected to a 2nd subculture. Larger and smaller trophic forms were again seen. The uneven staining and granular distribution were, for the larger forms, almost the same as in the first subculture but were less prominent in the case of the smaller form. The nuclear enlargement persisted but to a much lesser degree. Larger vacuoles were seen and the number remained in the region of 4 to 8. These trophic amoebae took 72 to 96 hours for encystment and cysts obtained were either large, small, or empty.

Effects seen at the 3rd subculture after treatment : (Figure IV).

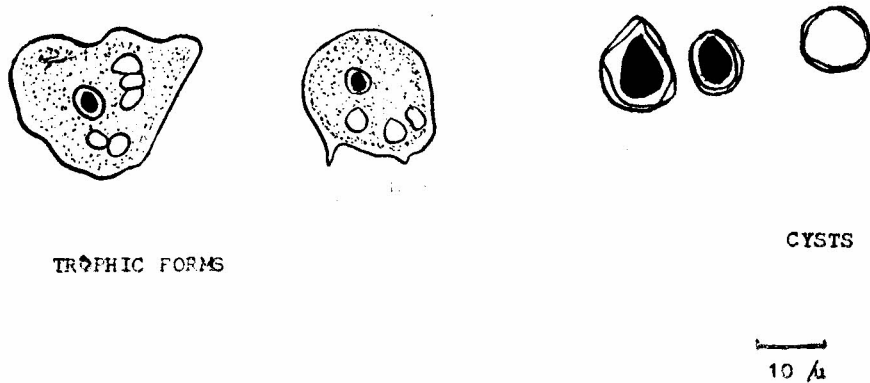


0.125% CYSTINE - THIRD SUB-CULTURE AFTER TREATMENT

CYSTINE TREATED AMOEBAE

Second subculture cysts were subcultured again. The new trophic forms showed an unmistakable evenness of the stain in the cytoplasm but a large number of them still had the peripheral hyaline bands. Precystic forms were visible even as early as 48 hours after emergence. The number of vacuoles had decreased and was now 3 to 6. Nuclear enlargement persisted but disappeared after about 72 hours. Encystment occurred after about 72 hours with large cysts and small being found. Some cysts were found attached to trophic forms. Completely empty cysts and those with some cytoplasmic material inside were also seen.

Effects seen at the 4th subculture after treatment : (Figure V).



0.125% CYSTINE - FOURTH SUB-CULTURE AFTER TREATMENT

The cysts obtained at the 3rd subculture were subjected to another subculture. The trophic forms that emerged showed a return to normality in the staining property of the cytoplasm and the granulation. The vacuoles were generally 3 to 6 in number. These trophic amoebae encysted within 72 hours. The shape size and nature of the cysts were variable, but there were fewer empty cysts than before.

Discussion

The nature of amino acid diffusion in the cell has been established by several workers (Danielli, 1952 ; Heinz 1954 ; Harris 1956). However it is not fully known what the essential amino acids required for this species of amoeba are though there have been many attempts to make axenic cultures (Lwoff, 1951). It has therefore been difficult to suggest the part Cystine plays in the formation of new material in the cytoplasm.

In the experiments described, the response of the trophic forms to the action of 0.125% Cystine has been striking. The normal period of encystment of trophic amoebae is within 48 hours but after the treatment this period has been extended even to 96 hours. There have also been some changes in the cytoplasmic pattern. The changes are of a temporary nature, their appearance and disappearance being seen when the animal passes through subsequent subcultures. A similar change obtained by us on a previous occasion is on record (Mookerjee & Waidyasekera, 1962).

Variation in the staining property of the cytoplasm is noticed in the 1st subculture. In the subsequent subcultures this unbalanced nature is shown by another variation in the cytoplasm namely the granulation. This leads us to the idea that the amino acid is taken in and stored in some form in the cysts. The variation in the basophilic reaction confirms this idea. Certain results of a similar nature were seen in the incorporation of amino acids in cytoplasm (Plaut & Rustard, 1956).

The changes seen in the structure size and number of vacuoles is also interesting. Changes in the vacuolar pattern upon chemical treatment have been reported in many experiments on amoebae. Vacuolar hyper-activity and its relationship to the cell body has been discussed in an earlier communication (Mookerjee & Waidyasekera, 1959) and the present observations confirm our conclusions.

Another phenomenon observed was the variability of the pattern of encystment upon treatment with the amino acid. This seems to be an attempt to avoid excessive Cystine.

A possible explanation of all the changes observed seems to be that the entry of Cystine into the cell body of the amoeba alters the balance in the 'amino acid pool' which then results in the formation of abnormal cytoplasmic end-products. The gradual return to normality in the course of several generations of encystment and subculture, seems to indicate the existence of some controlling mechanism in cytoplasmic nuclear relationship which tends to restore the balance in the 'amino acid pool' and to eliminate the abnormal end-products formed after treatment.

Summary

1. Action of 0.125% Cystine on the cell body of trophic amoebae has been investigated.
2. This percentage of Cystine brings forth various changes in the cytoplasm and the vacuolar structure.
3. Most striking are cytoplasmic changes like variation in the staining property or granulation of cytoplasm, vacuolar changes, and differences in cystic pattern.
4. These seem to be temporary effects and are got rid of in a majority while some disintegrate leaving empty cysts.

CYSTINE TREATED AMOEBAE

5. It seems that the entry of Cystine results in certain changes of cellular configuration which later return to normal through a feed-back mechanism probably involving nuclear-cytoplasmic relationship.

References

- Brachet, J., (1957) *Biochemical Cytology*, New York : Academic press Inc.,
- Christenson, H. N., et al (1952) Amino acid concentration by free cell neoplasm-relations among amino acids. *J. Biol. Chem.*, **198** ; 1-17.
- Danielli, J. F., (1952) Structural factors in cell permeability and secretion *Sym. Soc. Exp. Biol.*, **6**, 1-15.
- Gray, J., (1920) Relation of the animal cell to electrolytes II. The adsorption of hydrogen by living cells. *J. Physiol.*, **54**, 68-78.
- Harvey, E. N., (1911) Studies on the permeability of cells. *J. Expt. Zool.*, **10**, 507-555.
- Harris, E. J., (1956) *Transport and accumulation in Biological Systems*, London : Butterworths.
- Hazra, B., (1959) Species sentivity of soil amoebae to Trinitrophenol, *Natur wissenchyn*, **20**, 582-583.
- , (1961) Effect of different concentrations of Trinitrophenol on soil amoebae, *Proc Zool. Soc. Cal.*, **14** (1) 1-8.
- Heinz E., (1954) Kinetic studies on the influx of Glycine ICI4 intomouse ascites carcinoma cell, *J. Biol. Chem.*, **21**, 781-790.
- Lwoff, A., (1951) *Biochemistry and physiology of Protozoa, I & II*. New York: Academic Press.
- Lewis, M. R., (1923) Reversible gelation of living cells, *John. Hopk. Hosp. Bull.*, **34**, 373-374.
- Mookerjee S. & Waidyasekera, P. L. D., (1959) Surface layer in transporting vacuoles from the cell body, *Proc. Zool. Soc. Cal.*, **14** (1) 83-88.
- , (1962) Transient Physiological changes in Trypsinised amoebae. *Proc. Ind. Sci. Cong. Assoc.*, 1962.
- , Comparative study of outgrowths after amino acid treatment. (*in press*)

P. L. D. WAIDYASEKERA

- Mookerjee, S. & Alamelu, M. G., (1959) Nuclear cytoplasmic separation in N-Ethyl Urethane treated amoebae, *Nucleus*, **II (2)**, 215-222.
- Mookerjee, S. & Hazra, B., (1960) Pattern of substance localisation in the alternate existence in amoebae, *Proc. Nat. Inst. Sci.*, **26 (B2)**, 51-53.
- Phillipot, C. H., (1930) Effects of toxins and venoms on protozoa, *J. Expt. Zool.*, **56 (2)**, 177-183.
- Plaut, W. & Rustard, R. C., (1956) Uptake of Adenine-8-14-C by whole and fractional amoebae, *Nature*, **177**, 89-90.
- Singh, B. N., (1950) A culture method for growing small free living amoebae for the study of their nuclear division, *Nature*, **165**, 65-66.
- Tunicliff, R., (1928) Use of paramaecia for studying toxins and antibiotics, *Proc. Roy. Soc. Exp. Biol. Med.*, **26**, 213-217.
- Waidyasekera, P. L. D., & Mookerjee, S., Abnormal encystment after the action of certain amino acids (*in Press*).
- Zimmerman, A. M., (1959) Effects of selected chemical agents on amoebae, *Ann. N.Y. Acad. Sci.*, **78**, 631-646