Punjab Univ. J. Zool., Vol. 14, pp. 87-92, 1999

MITOSIS: A METHOD OF REPLICATION IN THE HAEMOCYTES OF COMMON GARDEN SNAIL FROM LAHORE, PAKISTAN

TANVEER, A., SHAHBAZ, K. AND KHAN, D.

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Abstract: Mitosis as a method of replication in molluscan haemocytes is being reported for the first time in common garden snail *Bensonies jacquemonti* from Lahore, Pakistan.

Key words: Land snail, haemocytes, mitosis.

INTRODUCTION

Good deal of disagreement on the origin, type and mode of replication of molluscan haemocytes has been reviewed by Cheng (1981), Cowden and Curtis (1981), Sminia (1981) and Bayne (1983). The molluscan haemocytes/blood cells are known to divide while they are in the haemopoetic tissue both *in vitro* (Bohuslav, 1933a,b; Bourne, 1935; Gatenby and Hill, 1934; Gatenby *et al.*, 1934) and *in vivo* (Lie and Heyneman, 1976; Lie *et al.*, 1975). However, Bohuslav (1933 and 1933a), Bourne (1935) and Gatenby (1932) observed no mitotic division in the cells of *Helix* maintained *in vitro*. However, mitotic cell division has been reported in the haemocytes of *Modiolus modiolus* (Gressen, 1937), *Philine aperta* (Fretter, 1937), *Crassostrea virginica* (Feng, 1967; Cheng and Rifkin, 1970; Feng *et al.*, 1971) and *Lymnaea rubiginosa* (Dondero *et al.*, 1977). Cytoplasmic fragmentation has been reported by Bourne (1935 in *Helix*) and Foley and Cheng (1972 in *Crassostrea virginica*). Proliferation of epithelial layers of various tissues and various parts of connective tissues has also been noted in *Helix pomatia* (Wagge, 1951, 1955), *L. stagnalis* (Müller, 1956), *Biomphalaria glabrata* (Pan, 1958), *Doto* sp. (Kress, 1968), *Bulinus* sp. (Kinoti, 1971) and *L. stagnalis* (Sminia, 1974). Endogenous budding as a method of replication in the haemocytes of *Angiospira alternata alternata* Say have also been reported by Khan (1986).

The present investigation is aimed to further investigate this method in a common land snail *Bensonies jacquemonti*.

0079-8045/99/0001-0087 $ 03.00/0

MATERIALS AND METHODS

Specimens of *B. jacquemonti*, collected from the Jinnah Garden, Lahore were maintained in the laboratory in large earthen pots half filled with humus soil at room temperature. The snails used in this study measured 1.80±0.25 mm - 3.0±0.55 mm in shell width. Their blood samples were obtained by inserting a micropippette directly into the heart after removing a piece of shell above this region (Guida and Cheng, 1980). The haemolymph was allowed to spread on glass slides for 30 minutes at room temperature and fixed in 1% glutaraldehyde in Sörensen's buffer (pH 7.4) at 5°C. These cells were stained with lead haematoxylin and basic fuchsin as described by Guida and Cheng (1980) and microphotographs were made at an enlargement of x1000.

RESULTS AND DISCUSSION

It was observed that 2-3% of the total blood cells of *B. jacquemonti* were binucleated and showed an unequal or equal amitotic division of the nucleus (Plate 1; Figs.1-4). These cells measured from 19-49 μm in diameter (29.21±22.14 μm). The mother nuclei measured 5-10 μm in diameter (9.34±1.89 μm) and daughter nuclei measured 6-11 μm in diameter (6.9±3.71 μm). The cell to nucleus ratio comes to 6:1 (Table I). Almost similar morphometric data has been reported by Khan *et al.* (1998) in this snail species.

In one of these daughter nuclei accumulates some portion of the cytoplasm cumulated from the parent cell and developed as an endogenous bud which then separated from the parent body. These buds immediately after separation were extremly small with a very poorly formed ectoplasm, a very high nucleus to cytoplasm ratio and showed limited pseudopodial activity (Plate 1, Fig. 5a) but they gradually grew into normal haemocytes, a sequence of this growth is shown in Plate 1, Fig. 5,a,b,c,d. It appears that although the question of a definite haemopoietic tissue still remains somewhat unsettled, there is ample proof to show that mature granulocytes and hyalinocytes undergo amitotic division and that new cells are formed through endogenous bud formation.

Although Feng *et al.* (1971) have reported the presence of fine structure like protocentrioles and associated microtubules in the mitotically dividing granulocytes of *Crassostrea virginica*. However, in the present investigation it was not possible to find out such details. Mitosis as a mode of cell division in molluscan haemocytes has been generally accepted by Malek and Cheng, (1974) and Sminia (1981). However, there are other workers who consider it as very rare phenomenon (Dondero *et al.*, 1977)

The presence of an amoebocyte producing organ between the pericardium and the posterior epithelium of mantle cavity has been suggested by Lie and Heyneman (1976) and Lie *et al.* (1976) with the emphasis that this organ became hyperactive during pathological conditions. Although their observations indicated that mature amoebocytes can generate daughter cells, special pool of stem cells or embryonic cells, as suggested by several other authors (Muller, 1956; Kinoti, 1971; Lie *et al.*, 1975).

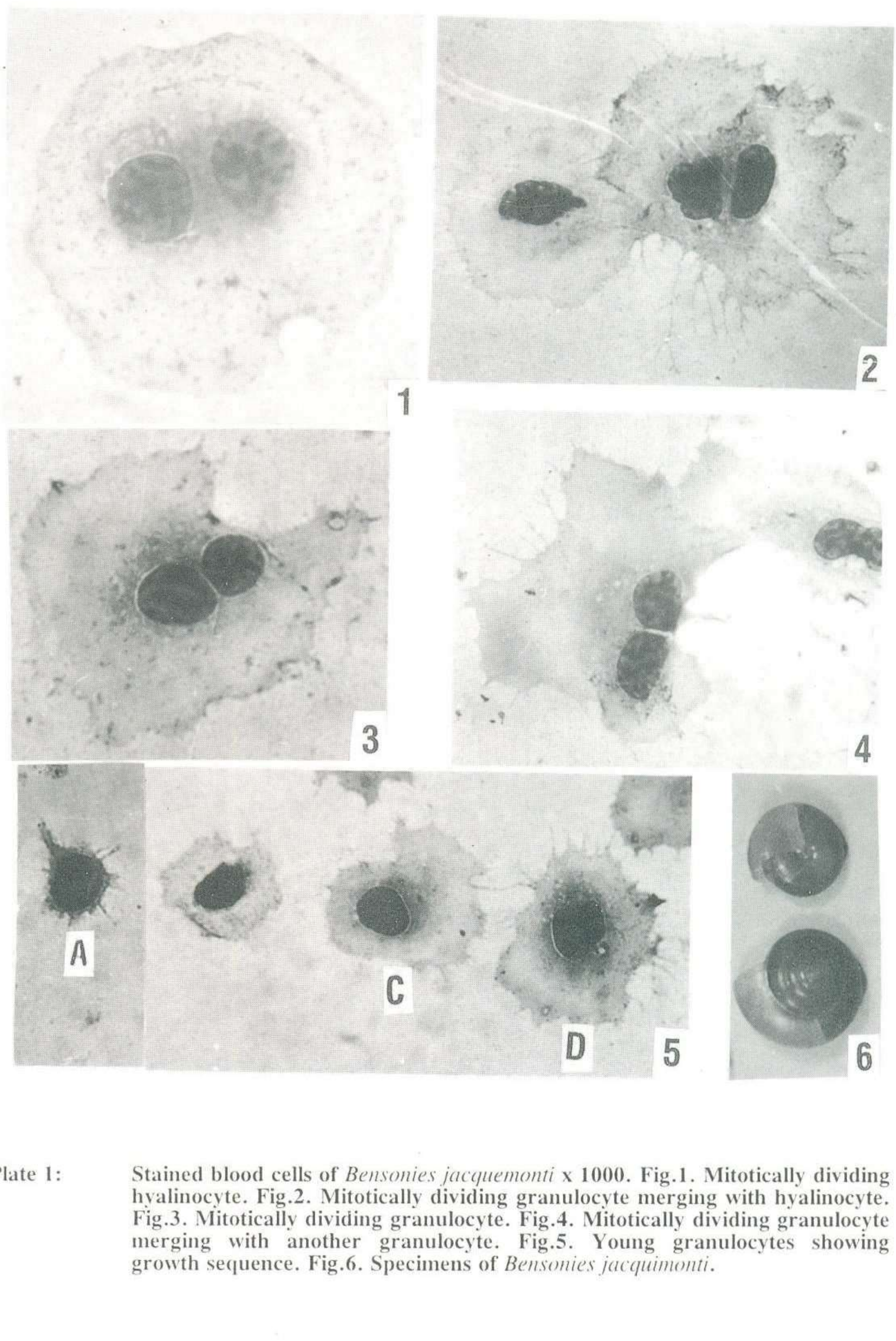

Plate 1: Stained blood cells of *Bensonies jacquemonti* x 1000. Fig.1. Mitotically dividing hyalinocyte. Fig.2. Mitotically dividing granulocyte merging with hyalinocyte. Fig.3. Mitotically dividing granulocyte. Fig.4. Mitotically dividing granulocyte merging with another granulocyte. Fig.5. Young granulocytes showing growth sequence. Fig.6. Specimens of *Bensonies jacquimonti*.

Table I: **Morphometric data (mean ± S.D.) of stained binucleated granulocytes of *Bensonies jacquimonte* (n=60).**

Cell		Mother nucleus		Daughter nucleus		Cell to mother nucleus ratio	Cell to dauthter nucleus ratio	Pseudopodia		
Diameter (μm) (Range)	Area $(\mu m)^2$ (Range)	Diameter (μm) (Range)	Area $(\mu m)^2$ (Range)	Diameter (μm) (Range)	Area $(\mu m)^2$ (Range)			Total number (Range)	Minimum length (Range)	Maximum length (Range)
29.21	1732.12	9.34	87.13	6.9	74.12	68:1	6:1	14.21	5.14	46.21
22.14	531.18	1.89	21.44	3.71	18.13			6.33	4.21	10.44
(19-49)	(390-1918)	(5-10)	(47-121)	(6-11)	(24-98)			(6-21)	(6-13)	(14-58)

REFERENCES

BAYNE, C.J., 1983. *Molluscan immunobiology* (Saleuddin, A.S.M. and Wilbur, K.M. eds). Vol. 4, pp. 407-486. Academic Press New York.

BOHUSLAV, P., 1933. Die Gewebezuchtung des postembryonalem verdauungstraktus der *Glandula salivalis* und des Receptaculum seminis bei Mollusken aus der Familic Helicidae. *Arch. Exp. Zellforsch Besonders Gewebzuecht*, **13**: 673-708.

BOHUSLAV, P., 1933a. Die Explanation des reinen postembrryonalen Herzbindegewebes aus *Helix pomatia*. *Arch. Exp. Zelforch Besondera Gewebezuecht,* **14**: 139-151.

BOURNE, G., 1935. Notes on some experiments with snail tissue in Culture, *Aust. J. Exp. Biol. Med. Sci.*, **13**: 43-48.

CHENG, T.C. AND RIFKIN, E., 1970. Cellular reactions in marine molluscs in response to helminth parasitism. In: *A symposium on disease of fishes and shell fishes* (S.F. Seniezku, ed.), Spec. Publ. No.5, pp. 443-496 *Amer. Fisher. Soc.* Washington, D.C.

CHENG, T.C., 1981. Bivalves. In "*Invertebrate blood cells*. Vol.I (eds. N.A. Ratcliffe and A.I. Rowley). pp. 233-300. Academic Press, New York, London.

COWDIN, R.R. AND CURTIS, S.K., 1981. Cephalopods. In "*Invertebrate Blood Cells*". Vol. I (N.A. Ratcliffe and A.F. Rowley, eds) Academic Press, New York, London.

DONDERO, JR., T.J., OW-YANG, C.K. AND LIE, K.J., 1977. Failure of irradiated *Echinostoma audyi* and *Hypoderaeum dingeri* to sensitize *Lymnaea rubiginosa* snails. *Southeast Asian J. Trop. Med. Pub. Hlth.*, **8**: 359-363.

FENG, S.Y., 1967. Responses of Molluscs to foreign bodies with special reference to oyster *Crassotrea gigas*. *J. Inv. Path.*, **12**: 53-63.

FENG, S.Y., FENG, J.S., BURKE, C.N. AND KHAIRALLAH, I.H., 1971. Light and electron microscopy of the leukocytes of *Crassostrea virginica* (Mollusca: Pelecypoela). *Z. Zellforsch. Mikroskop. Anat.*, **120**: 222-245.

FOLEY, D.A. AND CHENG, T.C., 1972. Interaction of Molluscs and foreign substances: the morphology and behaviour of hemolymph cells of the American oyster, *Crassostrea virginica, in vitro*. *J. invertebr. Pathol.*, **19**: 383-394.

FRETTER, G., 1973. The structure and function of the elementary canal of some Polyplacophora (Mollusca). *Trans. Roy. Soc. Edinburg*, **59**: 119-164.

GATENBY, J.B., 1932. Absence of mitosis in tissue culture land regeneration in *Helix aspersa*. *Nature (Lond.)*, 130-628.

GATENBY, J.B. AND HILL, J.C., 1934. Improved technique for nonaspetic tissue culture of *Helix aspersa* with notes on molluscan cytology. *Quart. J. Microsc. Sci.*, **77**: 129-155.

GATENBY, J.B., HILL, J.C. AND MACDOUGALD, T.J., 1934. On the behaviour of explants of *Helix aspersa* in aspectic and nonaspetic tissue culture. *Quart. J. Microsc. Sci.*, **77**: 129-155.

GRESSON, R.A.R., 1937. Studies on the cultivation of pieces of the mantle of *Modiolus modiolus*. *Quart. J. Microsc. Sci.*, **79**: 659-678.

GUIDA, V.G. AND CHENG, T.C., 1980. Lead Haematoxylin - Basic fuchsin: New stain for molluscan haemocytes. *Trans. Am. Micr. Soc.*, **99**: 135-140.

KHAN, D., 1986. Endogenous bud formation: a method of replication in molluscan haemocytes. *Pakistan J. Zool.*, **18**(4): 379-383.

KHAN, R.S., KHAN, D. AND TANVEER, A., 1998. Haemocytes of common land snail from Lahore, Pakistan. *Punjab Univ. J. Zool.*, **13**: 79-87.

KINOTI, G.K., 1971. I. Observations on the infections of *Bulinid* snails with *Schistosoma mansoni* II. The mechanism of resistance to infection. *Parasitology*, **62**: 161-170.

KRESS. A., 1968. Untersuchungen zur Histologie. Autotomie and Regeneration dreir *Doto, Doto coronata, D. pinnatifida D. fragilis* (Gestropoda : Opisthobranchiata). *Revue Suisse Zool.*, **75**: 235-303.

LIE, K.J. AND HEYNEMAN, D., 1976. Studies on the resistance in snails. 6. Escape of *Echinostoma lindolense* sporocysts from encapsulation in the snail heart and subsequent loss of the hosts ability to resist infection by the same parasite. *J. Parasite.*, **62**: 298-302.

LIE, K.J., HEYNEMAN, D. AND YAU, P., 1975. The origin of amoebocytes in *Biomphalaria glabrata*. *J. Parasitol.*, **61**: 574-576.

LIE, K.J., HEYNEMAN, D. AND JEOG, K.H., 1976. Studies on resistance in snails 4. Introduction of ventricular capsules and changes in the amoebocyte producing organ during sensitization of *Biomphalaria glabrata* snails. *J. Parsitol.*, **62**: 286-291.

MALEK, A.S. AND CHENG, T.C., 1974. Medical and economic malacology, New York.

MULLER, G., 1956. Morphologie lebensablauf and bildunsort der blutzellen von *stagnalis. L.Z. Zellorsch.*, **44**: 519-556.

PAN, C.T., 1958. The general histology and topographic microanatomy of *Australorbis glabratus*. *Bull. Mus. Comp. Zool. Harward Uni.*, **119**: 237-299.

SMINIA, T., 1974. Haematopoiesis in the fresh water snail *Lymnea stagnalis* studied by Electron microscope and autoradiography. *Cell Tiss. Res.*, **150**: 443-454.

SMINIA, T., 1981. Gastropods. In "*Invertebrate Blood Cells*" (N.A. Ratcliffe and A.F. Rowley, eds.) pp.191-232. Academic Press, New York.

WAGGE, L.E., 1951. The activity of the amoebocytes and the alkaline phosphatases during the regeneration of the shell in the snail *Helix aspersa*. *Quart. J. Microsc. Sci.*, **92**: 307-321.

WAGGE, L.E., 1955. Amoebocytes. *Int. Rev. Cytol.*, **4**: 31-78.

(Received: October 24, 1998)