FOLIA PARASITOLOGICA (PRAHA) 24: 377–380, 1977.

SHORT COMMUNICATIONS

EGG SHELL FORMATION IN THE TREMATODE DIPLODISCUS AMPHICHRUS — A CYTOCHEMICAL STUDY

U. KANWAR and M. AGRAWAL

Punjab University, Chandigarh

Abstract. In the ootype of the trematode *D. amphichrus* the oocyte along with one spermatozoan is first surrounded by a number of vitelline cells. Later on, an egg-shell is formed by the coalescence of vitelline globules. The egg shell is mainly composed of basic proteins rich in $-NH_2$ and -SS groups. In addition, a small amount of RNA is also detected in the shell material. The egg shells are not tanned and thus retain their staining behaviour even in the distal regions of the uterus. These non-tanned egg shells are presumed to be of keratin-type.

Trematodes, in general, produce large number of eggs which usually have well developed protective coverings. Various drugs are administered for killing trematode eggs inside the host as a measure for eradicating the parasitic infections. A knowledge pertaining to the chemical nature of the egg shell material is, therefore, essential for evolving methods and medicines for the control of trematode infections.

MATERIAL AND METHODS

The trematodes, *Diplodiscus amphichrus* (Tubangi, 1933), were collected from the rectum of the frog *Rana tigrina* Daud. These animals were fixed in various fixatives which include Zenker, formaldehyde calcium and weak Bouin's after chopping off their anterior and posterior suckers. The fixed material was processed according to the fixative used and embedded in paraffin wax (58°–60 °C) as well as in gelatine for different cytochemical tests. For the details of the latter, a reference may be made to Pearse (1968).

RESULTS

Egg shell formation in *D. amphichrus*, as in other trematodes, commences in the region of the ootype which is surrounded by Mehlis' gland cells. The oocytes are pushed into the lumen of the ootype. Each oocyte gets associated with about 10—12 vitelline cells and one spermatozoan and a layer is formed around whole of this mass which is then termed as an "egg" (Fig. 1). First of all, the Mehlis' gland cells pour off their lipoproteinous secretion into the lumen of the ootype (Kanwar and Agrawal 1974) which forms an outer preliminary membrane around the mass of cells. Each vitelline cell at this stage comprises a large number of vitelline globules arranged peripherally along with a condensed nucleus. These globules are duplex in nature. Their outer rims consist of lipids, carbohydrates, proteins and some traces of RNA, while the medullary

region is rich in proteins and contains a small amount of RNA (Kanwar and Agrawal 1976). It is in the ootype lumen that he vitelline globules in the vitelline cells move towards the periphery of egg (Fig. 2) and coalesce to form the shell inside the membrane formed by the Mehlis' gland secretion. It is observed that the shell is first formed in bits (Fig. 3) which later on coalesce and align themselves to complete the shell of the egg (Fig. 4).

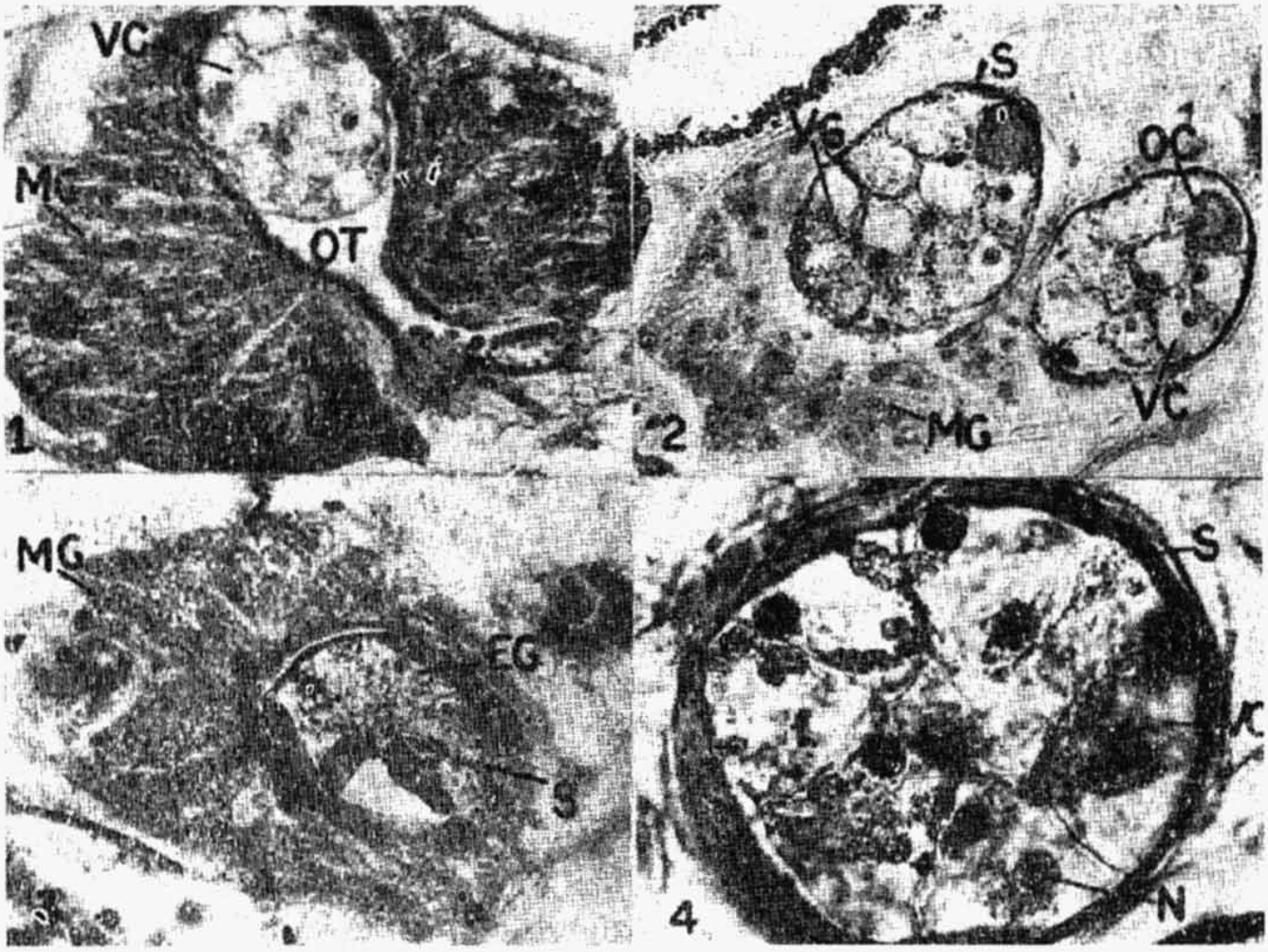

Fig. 1. Aggregation of vitelline cells in the ootype region. Zenker/mercurie bromphenol blue. (x 300)
Fig. 2. Shell in the process of formation by the peripheral movement and coalescence of vitelline globules. Zenker/methyl green/pyronin G. (x 300).
Fig. 3. Newly formed shelled egg in the ootype. Zenker/iron haematoxylin (x 300).
Fig. 4. Shelled egg in the distal region of the uterus. Zenker/iron haematoxylin (x 760).
EG—egg; Mg—Mehlis'gland; N—nucleus; OC—oocyte; OT—ootype; S—shell; VC—vitelline cell; VG—vitelline globules.

The newly formed shell is appreciably stained after mercuric bromphenol blue (Mazia et al. 1953) and ninhydrin-Schiff's (Yasuma and Itchikawa 1953) reactions revealing the presence of basic proteins in it. Tests for phenols are completely negative as is also the catechol incubation test of Smyth (1954). The shell reveals the presence of keratin (cystine) after permaganate-alcian blue and permaganate-aldehyde fuchsin tests of Madhavi (1971). It is slightly stained after methyl green/pyronin G test (Jordan and Baker 1955) (Fig. 2) thereby indicating the presence of RNA. Lipids and carbohydrates, however, do not enter the chemical make up of the shell. It remains transparent and non-tanned even in the distal parts of the uterus and retains its staining behaviour.

DISCUSSION

A number of studies have shown that the egg shells of trematodes, although appearing apparently similar in formation, show variation in their chemical nature. In most of the species so far studied, the amber coloured egg shells, whether thin or thick, are composed of quinone tanned protein-sclerotin (Stephenson 1947, Smyth and Clegg 1959, Burton 1963, Coil 1965, 1966, Kanwar et al. 1976). This tanned inelastic and highly resistant protein is formed from the precursors such as basic proteins, proteins rich in tyrosine and phenols which are produced in the shell granules of the vitelline cells. Evidence indicates that the phenolic compounds are oxidised by the enzyme phenolase (polyphenol oxidase, tyrosinase) to ortho-quinones which then react with free amino or sulphydryl groups on the adjacent protein molecules to bind the shell material together.

In the animal under investigation, *Diplodiscus amphichrus*, no phenolase could be detected in the shell globules by using DOPA-oxidase or catechol techniques (Kanwar and Agrawal 1976). Similarly, Smyth and Clegg (1959) could not demonstrate phenolase histochemically in *Gordoderina* sp., *Bucephaloides gracilenscens* and *Schistosoma mansoni*. Freeman and Llewellyn (1958) reported the same for *Proctoeces subtenuis*. Recently, Madhavi (1966, 1968) and Nollen (1971) denied the presence of phenolase in the shell globules of a number of digenetic trematodes that produce transparent and non-tanned eggs. Madhavi (1966, 1968), however, found these shell globules to be strongly positive to the test for the disulphide bonds leading her to identify the shell protein to be keratin.

During the present studies on *D. amphichrus*, the shell globules, as well as the shell, were found to give positive results for disulphide bonds with aldehyde fuchsin and alcian blue (after permaganate oxidation) methods of Madhavi (1971), thereby indicating the non-tanned egg shell to be of' keratin' type. Although Nollen (1971) has some doubts regarding the validity of these tests, it is conjectured that the egg shells of *D. amphichrus* consist of keratin-type protein.

Acknowledgements. The authors are thankful to Dr. G. P. Sharma, Professor and Head, Department of Zoology, Punjab University, Chandigarh for providing the necessary laboratory facilities.

ОБРАЗОВАНИЕ ЯЙЦЕВОЙ СКОРЛУПКИ У ТРЕМАТОДЫ *DIPLODISCUS AMPHICHRUS* — ЦИТОХИМИЧЕСКОЕ ИЗУЧЕНИЕ

У. Канвар и М. Агравал

Резюме. У оотипа трематоды *D. amphichrus* ооцит, одновременно с одним сперматозоидом, сначала окружен многочисленными желточными клетками. В дальнейшем скорлупка яйца образуется слиянием желточных гранул. Скорлупка яйца главным образом состоит из основных протеинов богатых группами $-NH_2$ и $-SS$. Кроме того в материале скорлупки было также обнаружено небольшое количество РНК. Яйцевые скорлупки несклеротизованы и поэтому сохраняют способность крашения даже и в дистальной области матки. Предполагается, что эти скорлупки кератинового типа.

REFERENCES

BURTON P. R., A histochemical study of vitelline cells, egg capsules and Mehlis' gland in frog lung fluke, *Haematoloechus medioplexus*. J. Exp. Zool. 154: 247—258, 1963.

COIL W. H., Observations on egg shell formation in *Hydrophitrema gigantica* Sandars, 1960. (Hemiuridae: Digenea). Z. Parasitenkd. 25: 510—517, 1965.

—, Egg shell formation in the notocotylid trematode, *Ogmocotyle indica* (Bhalerao, 1942) Ruiz, 1946. Z. Parasitenkd. 27: 205—209, 1966.

FREEMAN R. F. H., LLEWELLYN J., An adult digenetic trematode from an invertebrate host: *Proctoeces subtenuis* (Linton) from the lamellibranch *Scrobicularia plana* (Da Costa). J. Marine Biol. Assoc. United Kindgom 37: 435—457, 1958.

JORDAN B. M., BAKER J. R., A simple methyl green/pyridine technique. Quart. J. Micr. Sci. 96: 177—179, 1955.

KANWAR U., AGRAWAL M., Cytochemical studies on the Mehlis' gland cells of the trematode, *Diplodiscus amphichrus*. Zoologica Poloniae (In press).

—, —, Cytochemistry of the vitelline glands of the trematode, *Diplodiscus amphichrus*. Folia parasit. (Praha) (In press).

—, —, NATH V., Cytochemical studies on the egg shell formation in the trematodes, *Gastrothylax crumenifer* and *Ceylonocotyle dawesi*. Ress. Bull. Punjab Univ. (In press).

MADHAVI R., Egg shell in Paramphistomatidae (Trematoda: Digenea). Experientia 22: 93, 1966.

—, *Diplodiscus mehrai:* Chemical nature of egg shell. Exp. Parasitol. 23: 392—397, 1968.

—, Keratin in the egg shells of amphistomes. Histochemical differentiation from quinone tanned protein in other trematoda. Stain Technol. 46: 105—109, 1971.

MAZIA D., BREWER P. A., ALFERT M., Cytochemical staining and measurement of proteins with mercuric bromphenol blue. Biol. Bull. Woodsttole 104: 57—67, 1953.

NOLLEN P. M., Digenetic trematodes: Quinone tanning system in egg shells. Exp. Parasitol. 30: 64—72, 1971.

PEARSE A. G. E., Histochemistry Theoretical and Applied. J. A. Churchill London. Third ed., 1968.

SMYTH J. D., A technique for the histochemical demonstration of polyphenol oxidase and its application to egg shell in helminths and byssus formation in *Mytilus*. Quart. J. Micr. Sci. 95: 139—152, 1954.

—, CLEGG J. A., Egg shell formation in trematodes and cestodes. Exp. Parasitol. 8: 286—323, 1959.

STEPHENSON W., Physiological and histochemical observations on the adult liver fluke *Fasciola hepatica* L. III Egg shell formation. Parasitology 38: 128—139, 1947.

YASUMA Y., ICHIKAWA T. J., Lab. Clin. Med. 41: 296, 1953. (ex Pearse 1968).

Received 31 May 1976.

U. K., Punjab University,
Chandigarh, India