

THE CHEMISTRY OF THE MEMBRANES OF THE
EGG ENVELOPE OF *CRUZIA AMERICANA*
MAPLESTONE, 1930

(NEMATODA: KATHLANIIDAE)

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The Membranes of the Egg Envelope

It is now generally recognized that the shell or egg envelope of nematode eggs consists of three layers of different chemical composition. These layers are: 1) the inner vitelline membrane; 2) the true shell; 3) the outer protein layer.

Cruzia americana Maplestone, 1930 was redescribed by Crites (1956). In this nematode, the sequence of development of the egg and its membranes is as follows: The egg cells are produced from the germinal epithelium at the distal end of the ovary. As they pass down the ovarian tube, along the side of the rachis, each egg accumulates yolk material forming a vitellus. Sections through the distal part of the growth zone of the ovary show no membrane around the vitellus at this point. In the proximal end of the ovary the vitellus becomes pyramidal in shape, containing a germinal condensation and a nucleus. The egg at this point is surrounded by a definite membrane, the vitelline membrane (fig. 1 to 5).

After the egg has entered the oviduct, the vitelline membrane can be seen clearly surrounding the yolk. The ovum begins to assume the oval shape typical of the genus *Cruzia*. Whether this change in shape results from the contraction of the vitellus or is caused by action of the oviduct is not known (fig. 5 to 8).

Sperm penetration occurs in the oviduct close to the ovary. The shell begins to form immediately afterward. The shell forms first as a thin layer, but later it thickens from 1 to 3 μ as the eggs pass down the oviduct. The author believes that the shell is of endogenous origin.

The protein coat is absent in eggs in the oviduct. This external layer forms around the egg in the distal end of the uterus. This covering thickens slightly and becomes ridged as the egg moves farther down the uterus. The protein coat is probably exogenous, being formed from a secretion of the uterine wall. In preparations stained with either mucicarmine or Erlich's glycerine alum haematoxylin, the uterine wall shows droplets which stain with the same intensity as the protein coat of the egg. These stains, however, are not very specific, and this can be considered only as an indication that the outer coat is secreted by the uterine wall. The sculpturing of ridges in the protein coat is difficult to explain. Christenson (Chitwood and Chitwood, 1937) proposes that the mammillations of the eggs of *Ascaris* may be due to the basic principle of colloidal behavior. He believes that protein droplets accumulate around the shell, afterwards adhering and congealing and thus giving rise to a definite pattern. While the eggs are in the uterus, the vitellus shrinks away from the vitelline membrane leaving a fluid-filled cavity between it and the vitelline membrane, the perivitelline space. Walton (1924) reported that eggs of *Cruzia tentaculata* are oviposited while in a one-cell stage. The egg of *C. americana*, however, undergoes cleavage while in the uterus, and is oviposited while in a morula stage (fig. 11).

The Chemistry of the Egg Membranes

The chemistry of the egg membranes of *Cruzia americana* was investigated by using some of the tests applied by Chitwood (1938) and by Jones and Jacobs

(1939) to the egg membranes of other nematodes. These tests were supplemented by using histochemical methods. The materials used in the histochemical tests were either fresh and unfixed or fixed with a saturated solution of mercuric chloride. Tests were made at 23° C and 37° C, except when otherwise indicated.

The vitelline membrane.—(fig. 11). This membrane dissolved in absolute ether, chloroform, xylene, and acetone. It is insoluble in ten percent acetic acid, and is not dissolved by ten percent trypsin nor artificial gastric juice. The vitelline membrane is permeable to sodium hypochloride at 48° C and will melt when heated to 74° C. It is not stained by Sudan III or IV, but stains slightly with Sudan Black B. It does not dissolve in ten percent sodium-hydroxide or ten percent potassium-hydroxide. This membrane disappears, however, when the egg is heated to 160° C for 15 min in saturated potassium-hydroxide. The embryo inside the vitelline membrane does not stain when the eggs are treated with neutral red; but after treatment with fat solvents it stains readily with this dye. On the basis of these tests it is concluded that the innermost membrane is a lipid and probably a sterol or wax. Timm (1950) investigated the inner membrane of *Ascaris lumbricoides* var. *suis*, and on the basis of melting points concluded that it was a wax. He called this substance myricyl palmitate.

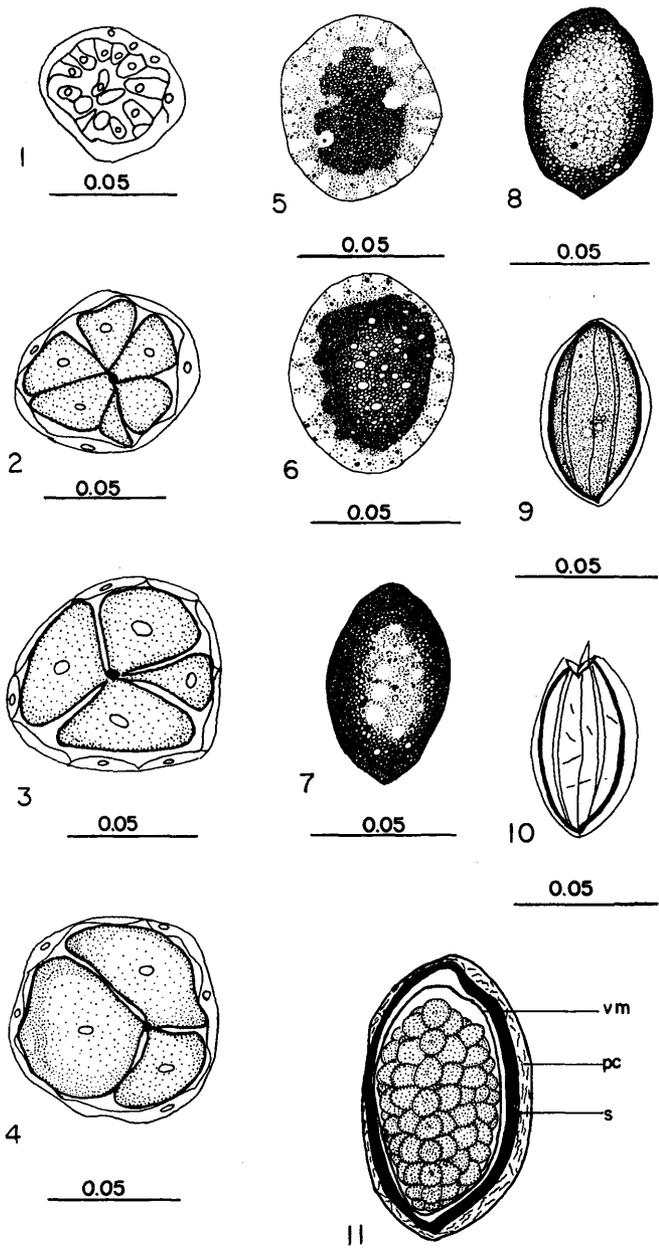
Shell proper.—The middle membrane, the shell, is insoluble in glacial acetic acid, ten percent sodium-hydroxide, and ten percent hydrochloric acid. It is not digested by artificial gastric juice. It gives a negative xanthoproteic test and a negative ninhydrin reaction. The shell dissolves in five percent sodium-hypochlorite in 30 min at room temperatures of 24 to 27° C. This membrane was tested for chitin, using the methods of Campbell (1929) and of Jones and Jacobs (1939). The eggs were heated in saturated potassium-hydroxide at 160° C for 15 min in a small, sealed capillary tube which was immersed in a glycerine bath. After such treatment, everything except the shell had disappeared. It stained brown in an iodine potassium-iodine solution, and it turned violet on the addition of dilute sulphuric acid. The shell dissolved in dilute acetic acid after treatment with potassium-hydroxide. The only substances known to withstand superheating with potassium-hydroxide are chitin and cellulose. In this case cellulose is eliminated, since cellulose is insoluble after treatment with potassium-hydroxide in dilute acetic acid. The shell stains a clear blue green with Alician blue, and it stains blue with Toluidine blue. It gives a negative Feulgen reaction, and stains purplish-red with periodic acid-Schiff (P.A.S.) treatment. These tests confirm the presence of carbohydrate material in the shell.

Eggs were removed from the ovarian end of the oviduct before the shell was formed. Some of these were submitted to the P.A.S. reaction and others to Alician blue. Both stains showed globules of carbohydrate material near the periphery of the vitellus. These globules appear in the very outer edge of the vitellus in eggs farther down the oviduct. They are much less numerous in eggs which have the shell completely formed, indicating that they probably give origin to the shell (fig. 5 to 9). Fauré-Frémiet believed that the egg shell of *Ascaris*

EXPLANATION OF FIGURES IN PLATE

(All scales in mm)

1. Ovary, germinal zone, cross section.
2. Ovary, distal end of the growth zone, showing developing ova around the rachis, cross section.
3. Ovary, cross section near middle of growth zone.
4. Ovary, proximal end of the growth zone, cross section.
- 5 through 9. These figures show the distribution of carbohydrate droplets in eggs from the upper oviduct to the uterus. Eggs from ovarian end of oviduct (fig. 5, 6). Eggs from uterine end of oviduct (fig. 7, 8). Egg from uterus (fig. 9).
10. Empty egg membranes, showing the rugosities and hatching orifice.
11. Morula stage, VM, Vitelline membrane; S, Chitinous shell; PC Protein coat.



lumbricoides was formed endogenously from glycogen. Alician blue shows only the presence of polysaccharides, and it is not specific for glycogen. The P.A.S. reaction gave a bright red color for glycogen, and the globules mentioned above stained purplish-red with this reaction. On the basis of the above tests it is concluded that the shell of the egg is chitin and that it is probably formed endogenously from carbohydrate materials in the vitellus; however, there is no positive evidence that this carbohydrate is glycogen.

The protein coat.—This outer coat dissolves in artificial gastric juice in three hours at room temperatures of 24° to 27° C. It dissolves in ten percent trypsin and also in one percent hydrochloric acid. It swells, but does not dissolve, in dilute acetic acid. One to three percent potassium-hydroxide dissolves the outer coat. It also is dissolved in picric acid. The membrane is not soluble in water. This outer coat gave a positive xanthoproteic test, turning orange in the presence of ammonia after being treated with warm nitric acid; it gave a ninhydrin test only with unfixed eggs, and even then only a slight diffuse blue color; and it turned orange when subjected to Millon's reagent. All of these tests indicate that this outer coat of the egg of *C. americana* is protein in nature.

On the basis of his findings, Chitwood (1938) concluded that the outer membrane of *Ascaris* eggs was not an albumin, collagen, fibroid, or keratin. He presumed that it might be a conjugated protein such as a mucoid. Wottage concluded, on the basis of staining results with Sudan III, that some lipoidal material is also present in this membrane in the eggs of *Ascaris*. The eggs of *C. americana* were tested further with histochemical test to check these possibilities. The outer membrane gave a negative Feuglen reaction, but it stained a light pink with the P.A.S. reaction, and also with mucicarmine. Toluidine blue gave a doubtful test on eggs fixed with mercuric chloride, the membrane sometimes staining a clear green and sometimes giving shades of rose. The membrane stained very lightly with Sudan III. On the basis of these tests, it was concluded that the outer coat of the eggs of *Cruzia americana* is probably a mucoprotein and that some lipoidal substances are present.

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