Histochemical Demonstration of Vitamin C in *Hymenolepis nana* var. *fraterna*

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The presence of ascorbic acid in *Hymenolepis nana* var. *fraterna* has been demonstrated by a series of cytological techniques based on the classical acetic-silver nitrate technique of Giroud and Leblond, 1934 (Comptes Rendus de la Societe de Biologie, 115:705). This consists essentially of successive treatment of the organisms in acetic-silver nitrate, sodium sulphite, sodium thiosulphate, and distilled water in the dark at room temperature. A dark green filter was employed to facilitate handling of the worms under these conditions. The presence of the reduced form of ascorbic acid is indicated by precipitates of reduced silver deposited in the tissues. Sodium thiosulphate was necessary to remove the silver chloride, which may be formed by the action of silver nitrate upon cellular chlorides. The inhibition of a black precipitate of silver sulphide, produced by the action of sodium thiosulphate upon silver nitrate, takes place by the addition of sodium sulphite prior to the thiosulphate. Immersion time intervals, as well as concentrations of the above compounds, were varied. A freezing technique, as outlined by Glick, 1949 (*in* Techniques of Histo- and Cytochemistry, Interscience Publishers, N. Y.) was also employed. The validity of the acetic-silver nitrate technique has been reviewed by many authors, outstanding perhaps, are the reviews of Bourne, 1936 (Anatomical Record 66:369), and Sosa, 1952 (Experimental Cell Research 31(1):184). Some of the infected mice were on a normal diet, while others were on an ascorbic acid supplemented diet prior to the determinations.

Whole mounts and serial sections of Hymenolepis showed the presence of dark bodies, suggesting the presence of reduced ascorbic acid. Particles in the younger proglottids appeared as dense reticuli, and as fine black granules distributed irregularly in the cytoplasm. Granules were also observed in the oncospheres. The appearance of the reticuli suggested the configuration of the Golgi apparatus.

Bourne, 1951 (Cytology and Cell Physiology, Oxford at the Clarendon Press, London) and others have demonstrated that a topographical coincidence exists between the Golgi apparatus, and vitamin C. Bourne, in some cases, demonstrated the presence of ascorbic acid in the internal region of the mitochondria. Sosa, (loc. sit.) contests this, and claims that the accumulation of the granules in a juxtanuclear zone, may not necessarily mean their localization inside the Golgi apparatus, or even inside the Golgi zone. He disagrees also with Hirsch's theory of the active part taken by the Golgi apparatus in ascorbic acid storage in the cell, and points out that there is no method for simultaneously and completely revealing the Golgi apparatus and the vitamin C granules.

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The presence of vitamin C in this organism is of interest since mice are known not to require ascorbic acid in their diet, under normal conditions. Four possibilities are suggested as to the vitamin source in this organism. The parasite may be getting this substance from 1) the host's diet as is the case for riboflavin (Addis and Chandler, 1944; Journal of Parasitology 30:369); 2) bacteria in the intestine since bacteria do not appear to require vitamin C; 3) the mucosa of the host as is the case with vitamins A, B_1, D, and E (Addis and Chandler (loc. cit.); or 4) the parasite may be synthesizing vitamin C, which may be possible since ascorbic acid synthesis is dependent on the products of glucose metabolism (Roy and Guha 1946, Nature 158:238).

Since vitamin C possesses strong reducing powers, and is capable, with glutathione, of forming an oxidation-reduction system, its possible value as a respiratory mechanism in this tapeworm is readily recognized.

A micro-chemical determination for ascorbic acid, employing the photometric indophenol-xylene procedure, will be undertaken to substantiate the above results, and determine the percentage of vitamin present.