Percentage inhibition of brain serum, and erythrocytes acetylcholinesterase and liver succinic dehydrogenase activities after Cyolane administration [a]

Enzyme	Daily dose (mg/kg)	Percentage inhibition [b]		
		1 week	2 weeks	4 weeks
Brain acetylcholinesterase	0.9 [c]	54 ± 3.64	—	—
	0.18	40 ± 3.23	36 ± 3.3	35 ± 3.28
	0.09	28 ± 2.83	15 ± 2.88	16 ± 2.33
	0.045	0	0	0
Erythrocytes acetylcholinesterase	0.9 [c]	37 ± 3.75	—	—
	0.18	15 ± 3.28	40 ± 3.75	45 ± 2.33
	0.09	0	28 ± 2.31	30 ± 2.88
	0.045	0	10 ± 2.19	10 ± 1.87
Plasma acetylcholinesterase	0.9 [c]	28 ± 1.04	—	—
	0.18	5 ± 1.87	44 ± 3.3	42 ± 3.64
	0.09	0	30 ± 1.9	30 ± 2.48
	0.045	0	15 ± 2.54	17 ± 1.87
Liver succinic dehydrogenase	0.9 [c]	0	—	—
	0.18	0	37 ± 1.73	40 ± 1.27
	0.09	0	30 ± 2.71	35 ± 1.38
	0.045	0	12 ± 2.54	15 ± 1.78

[a] Data are means of 4–6 rats. [b] Mean ± S.D. [c] Rats treated with 0.9 mg/kg died within 10 days.

corresponding to 0.9, 0.18, 0.09, and 0.045 mg/kg (1/10, 1/50, 1/100 and 1/200 of the LD_{50} respectively, for 1, 2 and 4 weeks. Rats were sacrificed 24 h after the last dose. Blood was collected in centrifuge tubes, and brain and liver were quickly removed for enzymatic assay.

Acetylcholinesterase in brain was estimated after the method of HESTRIN[11], using 10% rat brain homogenate and an incubation period of 15 min. Acetylcholinesterase in erythrocytes and plasma were assayed according to MICHEL[12]. Succinic dehydrogenase activity was measured colorimetrically using 0.5% solution of 2-3-5-triphenyl-tetrazolium chloride according to the method of FAHMY[13].

Results and discussion. In the present experiments, repeated short-term administration of Cyolane to rats resulted in a decrease in acetylcholinesterase activity of brain, erythrocytes and plasma, (Table). This inhibition was comparable to other organophosphorus pesticides[2–6].

The inhibition of the enzymes under investigation remained almost constant from 2 up to 4 weeks. It seems that the cumulative effects with repeated administration of low doses were compensated physiologically[2].

The extent of inhibition of acetylcholinesterase and succinic dehydrogenase was proportional to the Cyolane dose. This proportionality does not seem to exist between the percentage inhibition and the administration period[14].

Zusammenfassung. Das Phosphorsäureester-Insektizid Cyolan hemmt nicht nur die Cholinesterasen, sondern auch die Succinodehydrogenase der Leber.

M. R. E. BAHIG

Department of Radioisotopes, Atomic Energy Establishment, Cairo (U.A.R.), 3 January 1975.

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An Autoradiographic Demonstration of Blood Cell Renewal in *Styela clava* (Urochordata: Ascidiacea)

The blood cells of ascidians circulate in the blood channels and wander throughout the tissues and the tunic. Although some blood cell types are common to all ascidians, other blood cell types often differ from species to species. The number of blood cells described in any one species also varies with the morphological criteria of the authors. With light microscopy, 8 types have been described in *Styela clava*[1] while 5 types have been described in *Styela plicata*[2].

The origin and renewal of ascidian blood cells have been the subject of controversy. The neural gland[3] and haemoblasts in the connective tissue[4] have been reported as sites of blood cell formation. Several authors have

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remarked on the absence of mitotic figures in the blood spaces[5,6]. There is general agreement that the lymphocyte is the progenitor blood cell type. Whether lymphocyte cell division occurs only in the lymph nodules of the body wall and digestive tract[6] or both in the lymph nodules and in the circulating blood[1,2] has not been established. In either case, the lymphocytes are presumed to differentiate into the other cell types by the loss of the nucleolus, an increase in the amount of cytoplasm, and the acquisition of various cytoplasmic inclusions and vacuoles. The transformations of one blood cell type to another have been deduced from morphological criteria alone[2,7].

In the present investigation, autoradiography with tritiated thymidine was used to localize sites of blood cell proliferation and determine possible blood cell transformations in the ascidian *Styela clava*. A short exposure to tritiated thymidine labeled blood cells engaged in premitotic DNA synthesis. By sampling tissues at increasing time intervals after this short exposure, the fate of the proliferating cells was followed.

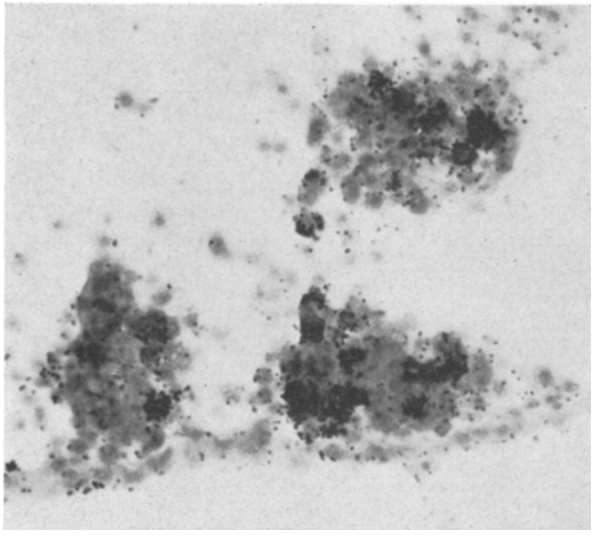

Fig. 1. An autoradiogram of the lymph nodules in the body wall of *Styela clava* 1 h after the injection of tritiated thymidine. ×600.

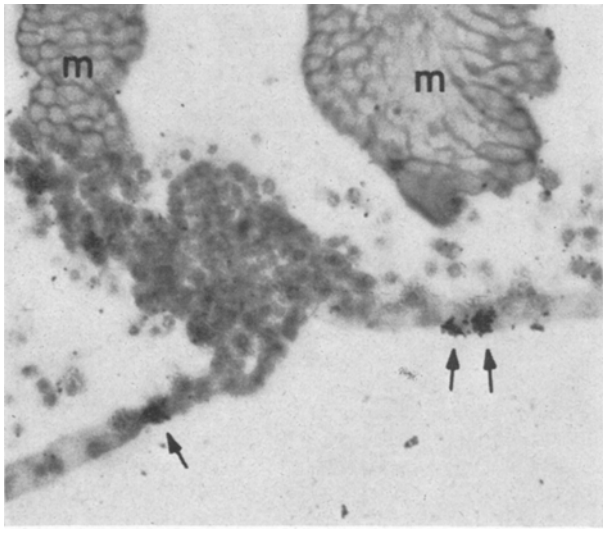

Fig. 2. An autoradiogram of the lymph nodules in the body wall of *Styela clava* 60 days after the injection of tritiated thymidine. The blood cells are no longer labeled although several atrial epithelial cells (arrows) are still labeled. m, muscle. ×600.

Materials and methods. Specimens of *Styela clava* were collected from Mission Bay, San Diego, California, USA and injected intra-atrially with 1 μCi of tritiated thymidine (New England Nuclear Corp.) per g fresh weight. The aqueous solution of tritiated thymidine (specific activity 6.7 Ci/m*M*) was diluted with an equal volume of 2 times concentrated sea water before use. 3 individuals were sacrificed by fixation in Bouin's fluid at each of the following time intervals: 1 h, 10, 20 and 60 days. The body wall and digestive tract were dissected out, dehydrated, and embedded in paraffin. 7 μm sections were covered with Kodak Nuclear Track Emulsion type NBT-2 by the dipping method and stored at 4 °C for periods of 2 weeks to 2 months. Autoradiograms were developed in Kodak D-19 developer (3 min), and sections were stained through the emulsion with hematoxylin.

Results. 3 types of blood cells can be recognized in autoradiograms: the lymphocyte, the leucocyte, and the vacuolated cell. All 3 blood cell types occur free in the circulating blood and clustered in lymph nodules within the connective tissue of the body. In the body wall, the lymph nodules occur in patches immediately adjacent to the atrial epithelium (the internal lining of the body wall). In the digestive tract, they are most concentrated in the branchial wall.

Lymphocytes are small, round to oval cells approximately 6–8 μm in diameter with a large nucleolated nucleus. The nucleus fills most of the cell and is surrounded by a small amount of basophilic cytoplasm. Several lymphocytes frequently cluster together in the interior of the lymph nodules. Cell boundaries are difficult to distinguish as the large basophilic nuclei of adjacent cells crowd each other with little intervening cytoplasm.

Leucocytes are large blood cells about 10–14 μm in diameter with a small, basophilic nucleus which is eccentrically displaced and lacks a conspicuous nucleolus. Most of the cell is filled with slightly basophilic cytoplasm. Leucocytes range in shape from spherical to oval and may have several cytoplasmic processes. The cytoplasm may be granular or transparent and may contain a large basophilic inclusion. Leucocytes probably include several cell types which are difficult to distinguish from each other. In the lymph nodules, the abundant cytoplasm around each nucleus produces regions of light basophilia and makes the leucocyte nucleus easily distinguished from the lymphocyte nucleus.

Vacuolated cells are the largest of the blood cells, being roughly 16–18 μm long. The nucleus is eccentrically placed and lacks a conspicuous nucleolus. Within the cytoplasm, the vacuolated cells contain numerous vacuoles which are clear or yellow in hematoxylin stained sections and which have a high index of refraction. Vacuolated cells frequently lie embedded between the atrial epithelium and the connective tissue of the body wall.

A 1 h exposure to tritiated thymidine labeled many blood cells in the body. Blood cells were labeled both in the lymph nodules and in the blood channels. In the lymph nodules, both lymphocytes and leucocytes were labeled (Figure 1), and labeled nuclei were frequently clustered in small groups. Probably no vacuolated cells were labeled at this time; however, positive reactions were difficult to detect in autoradiograms as the vacuoles appear very similar to out-of-focus silver grains.

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By 20 days after injection, most of the labeled cells in the lymph nodules occurred in the peripheral parts of the nodules. Some vacuolated cells were now unequivocally labeled, but few lymphocytes were labeled any longer. Most of the labeled cells, both in the lymph nodules and in the circulating blood, were leucocytes and vacuolated cells. At 60 days after the administration of tritiated thymidine, most of the blood cells in the lymph nodules were unlabeled (Figure 2). However a few scattered labeled leucocytes and vacuolated cells occurred in the connective tissue below the atrial epithelium and in the blood channels.

Discussion. The blood cells of *Styela clava* constitute a renewing[8] cell system with a renewal time on the order of several weeks. As suggested by other authors[1,2], blood cell proliferation occurs both in the lymph nodules and in the blood channels. The possible blood cell transformations are shown in Figure 3. Although both lymphocytes and leucocytes proliferate, the lymphocyte is probably the more primitive blood cell type. It is ultrastructurally the most undifferentiated blood cell type[9] and may also be capable of differentiating into germinal cells[10] and somatic cells other than blood cells[11]. Presumably the lymphocytes differentiate into leucocytes. However, since the leucocytes divide and also probably represent several separate cell types, the leucocytes themselves might be composed of stem, dividing transit, or non-dividing transit[12] components. Since the differentiated vacuolated cells do not divide, they must be differentiating from a precursor cell type; whether this precursor is a lymphocyte or a leucocyte could not be determined in the present investigation. Based upon morphological criteria, however, vacuolated cells have been reported to differentiate from intermediate cell types and not from lymphocytes in other ascidians[7,13].

Blood cells are renewed in insects[14] and mammals[15] but not in echinoderms[16] where blood cells have characteristics of expanding[8] cell populations. In mammals, proliferating cells are most concentrated in the bone marrow, lymph nodes, and spleen, but, like in *Styela*, they also occur in the circulating blood and in the connective tissue. In *Styela*, other elements of the vascular system (the heart and connective tissue lining the blood channels) comprise expanding cell populations[17].

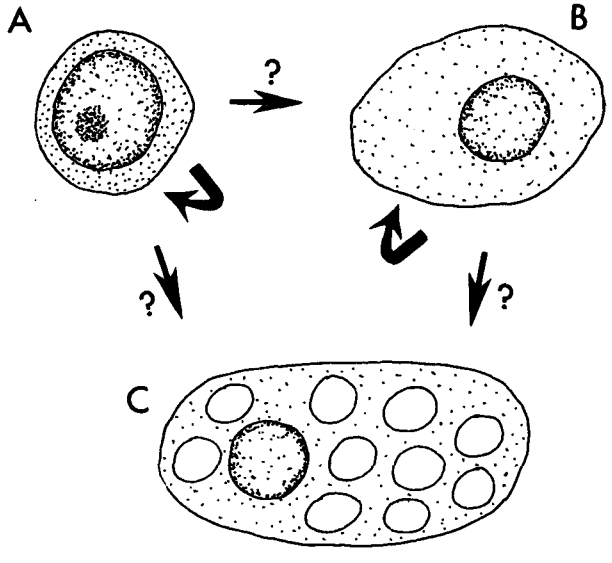

Fig. 3. Transformation of blood cell types in *Styela clava*. A, lymphocyte; B, leucocyte; C, vacuolated cell.

Summary. The blood cells of *Styela clava* were shown by autoradiography with tritiated thymidine to be renewed after several weeks. Proliferating lymphocytes and leucocytes occurred in the lymph nodules and blood channels of the body. Vacuolated cells did not proliferate but differentiated from a precursor cell type.

T. H. Ermak[18]

Division of Marine Biology,
Scripps Institution of Oceanography,
La Jolla (California 92037, USA), 5 March 1975.

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Aggregations of Dense Granules in Mitochondria of Active Pulmonary Lymphatic Endothelial Cells

In a previous study[1] we investigated the rôle of the peribronchovascular lymphatics in the clearance of intratracheally instillated ferritin and carbon particles. Both tracers reached the lymphatic lumen mainly via the open intercellular junctions. Ferritin particles were moreover absorbed by the endothelial cells and accumulated, probably to be digested[2], in secondary lysosomes.

The purpose of the present study was to investigate if these activities of the lymphatic endothelial cells are associated with morphological changes of their mitochondria, as it is well known that more active mitochondria display an altered fine structure[3].

We now demonstrate aggregations of small, dense, and more or less rounded granules (300 à 800 Å) occurring in mitochondria of pulmonary lymphatic endothelial cells, which had endocytosed ferritin, were fixed in a mixture of glutaraldehyde and osmium tetroxide, and stained with uranyl acetate and lead citrate. Although the precise nature of these granules, which have not been reported before to the best of our knowledge, remains unexplained, it is suggested that these aggregations are related to an in-

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