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A General Model for Leucocyte Cell Renewal in Bivalve Mollusks

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INTRODUCTION

It is not the intent of this paper to survey every study or discuss every hypothesis that has been formulated about blood cell renewal in bivalve mollusks; it is sufficient to state that most hypotheses have not been based on any substantial experimental evidence. Nor is it my intent to review the confusion and controversy that has circumfused molluscan blood cell terminology. I will use the term "leucocyte" when referring to molluscan blood cells except when specific cell types are being discussed (the reader is directed to Cheng and Rifkin, 1970, for an interesting discussion about the use of the term in molluscan hematology).

The purpose of this report is to describe a model of the normal leucocyte cell renewal system (CRS) that is based primarily on research conducted during the past 6 years by a number of workers. Unpublished findings from our (Mix and Tomasovic) studies using high specific activity tritiated thymidine, ^{3}H-TdR, (Mix and Tomasovic, 1973) in analyzing leucocyte renewal in *Ostrea lurida* will also be included (see Mix and Tomasovic, 1973; Tomasovic and Mix, 1974; Mix, 1975a, for a description of the methods).

It is now generally accepted that there are two common types of leucocytes found in bivalve mollusks, hyalinocytes (Foley and Cheng, 1972) and granulocytes (Galtsoff, 1964; Tripp, Bisignani, and Kenny, 1966; Farley, 1968; Cheney, 1971; Feng, et al., 1971; Ruddell, 1971a, b, c; Foley and Cheng, 1974; Cheng, 1975) (Fig. 1). Other types, fibroblasts (Pauley and Sparks, 1966, 1967; Rifkin and Cheng, 1968; Cheney, 1969; DesVoigne and Sparks, 1969; Cheng and Rifkin, 1970; Ruddell, 1971c; Sparks, 1972), fibrocytes (Foley and Cheng, 1972; Cheng, Cali, and Foley, 1974; Cheng, 1975), myoblasts (Ruddell, 1971c), and pigment cells (Stein and Mackin, 1955; Galtsoff, 1964; Haigler, 1964; Cheney, 1969; Ruddell and Wellings, 1971) have been described although there is considerable confusion and disagreement about their structure, function, and origin. For details on what is known about the structure and function of the various molluscan blood cells, see Cheng and Rifkin (1970), Sparks (1972), and Cheng (1975) for useful reviews.

Finally, it must be emphasized that the proposed leucocyte renewal model is a simple generalized model intended to describe a basic process that unquestionably occurs in all bivalves. It is obvious that variations will be (have been) found between bivalves of different subclasses and possibly even genera. Nevertheless, it is the author's judgment that sufficient studies have been conducted on enough different bivalve species to lend considerable support to the proposed model. The reader is referred to Fabrikant (1972) for a review of terminology used in this report.

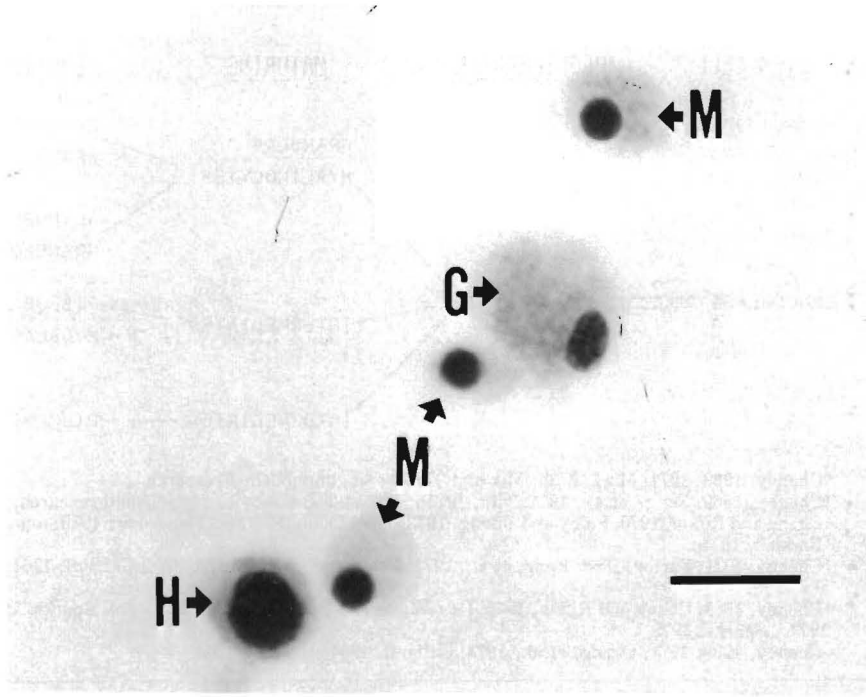


Figure 1.—Granulocytes and a young hyalinocyte from *Ostrea lurida*. G = mature granulocyte; M = young, maturing granulocyte; H = a young hyalinocyte. Bar = 5 μm.

LEUCOCYTE CELL RENEWAL

Cheney (1969), from studies utilizing H-TdR and autoradiographic analysis, proposed the following model for the development of leucocytes in the Manila clam, *Tapes semidecussata:* A hypothetical stem cell (a leucoblast or perhaps a fibroblast) divides and gives rise to a dividing population of hyalinocytes with nuclei of different diameters but usually greater than 3 μm. After one or two divisions (perhaps more) these cells enter a nondividing population and mature into phagocytes (he described only acidophilic granulocytes). He felt it was possible that the pigment cell was the final cell type and was in essence an aged acidophilic granulocyte.

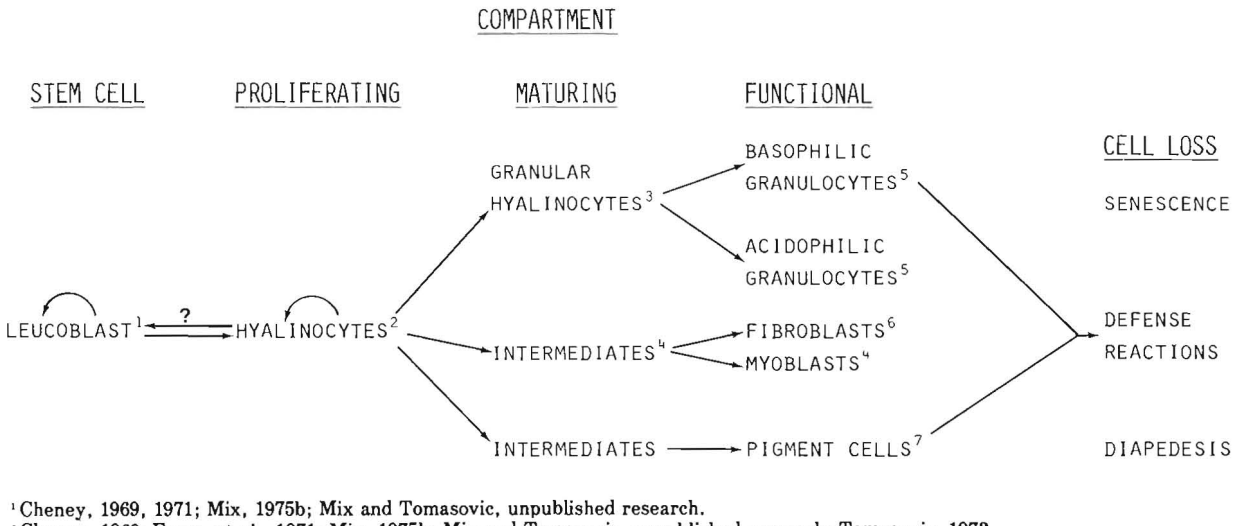
Since 1970, several studies (Cheng and Rifkin, 1970; Cheney, 1971; Feng, et al., 1971; Ruddell, 1971a, b, c; Foley and Cheng, 1972, 1974; Sparks, 1972; Mix, 1975a; Mix and Tomasovic, unpubl. report) have provided additional information about leucocyte renewal. Studies in which ^{3}H-TdR has been used (Cheney, 1969; Mix and Tomasovic, 1973; Tomasovic, 1973; Mix, 1975a; Mix and Tomasovic, unpubl. report) are, of course, particularly important. The proposed model is summarized in Figure 2. For convenience, each compartment will be considered separately.

Stem Cell Compartment

Little is known about the hypothetical stem cell. Cheney (1971) reported that the largest leucocyte in the Manila clam was similar to the vertebrate hemocytoblast and had a nuclear diameter of 6-8 μm. He felt (Cheney, 1969) that such a cell may be the stem cell, or leucoblast, of the leucocyte CRS. He also mentioned (Cheney, 1969) it was possible that a fibroblast or even a dedifferentiated gastric epithelial cell may be the stem cell but felt the latter was an unlikely source. We (Mix and Tomasovic, 1973; Tomasovic, 1973; Mix, 1975a; Mix and Tomasovic, unpubl. report) have observed large undifferentiated cells in both *O. lurida* and the freshwater mussel, *Margaritifera margaritifera*, that are consistently labeled (they are often the only cell type labeled) within hours after injection with ^{3}H-TdR (Fig. 3). This observation indicates that it probably has a relatively short cell cycle time and their frequent proximity to labeled hyalinocytes may suggest they are leucocyte stem cells. The large cells are located throughout the loose connective tissue but are most common in areas underlying the mantle. Cheney (1969) made similar observations about such cells in *T. semidecussata*. It is proposed that the large cell may be the leucocyte stem cell and suggest that "leucoblast" (after Cheney, 1969) is an appropriate term for describing it. It may be significant that there is good evidence for a single multipotential hemopoietic stem cell in vertebrates (Miale, 1972).

Proliferation Compartment

There is little doubt that hyalinocytes are members of the P compartment (Fig. 4) and mitotic division of these cells serves to amplify the number of cells which enter the M compartment (Cheney, 1969; Feng, et al., 1971; Ruddell, 1971b; Mix, 1975a; Mix and Tomasovic, unpubl. report). Interestingly, this possibility was described at the beginning of this century (Kollman, 1908). It is not known how many divisions occur before the hyalinocyte enters the M compartment. Two factors inhibit the resolution of this problem: The ^{3}H-TdR label may



[1] Cheney, 1969, 1971; Mix, 1975b; Mix and Tomasovic, unpublished research.
[2] Cheney, 1969; Feng, et al., 1971; Mix, 1975b; Mix and Tomasovic, unpublished research; Tomasovic, 1973.
[3] Cheng and Rifkin, 1970; Foley and Cheng, 1972; Mix, 1975b; Mix and Tomasovic, 1973, unpublished research; Tomasovic and Mix, 1974.
[4] Ruddell, 1971c.
[5] Cheney, 1971; Farley, 1968; Feng, et al., 1971; Foley and Cheng, 1972, 1974; Galtsoff, 1964; Mix, 1975b; Ruddell, 1971a, b, c; Tripp, et al., 1966.
[6] Cheney, 1969; Cheng and Rifkin, 1970; DesVoigne and Sparks, 1969; Pauley and Sparks, 1966, 1967; Rifkin and Cheng, 1968; Ruddell, 1971c; Sparks, 1972.
[7] Cheney, 1969, 1971; Cheng, et al., 1974; Galtsoff, 1964; Haigler, 1964.

Figure 2.—A simple, generalized model of bivalve molluscan leucocyte renewal. Arrows indicate the flow of developing cells from one compartment to the other. Footnotes refer to appropriate references.

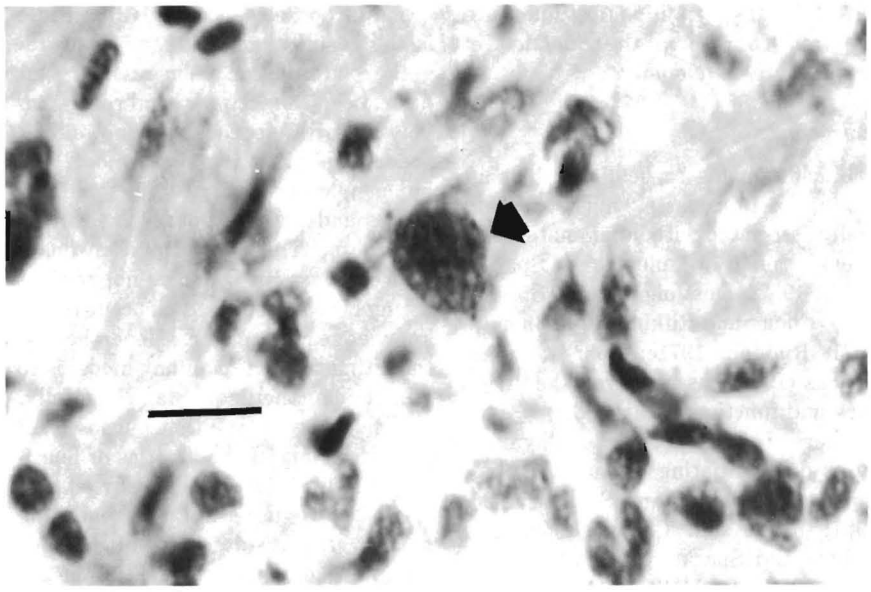


Figure 3.—A large leucocyte, thought to be a leucoblast labeled with tritiated thymidine, in the connective tissue underlying the mantle in *O. lurida*. The developed grains have obscured the nuclear details. Bar = 10 μm.

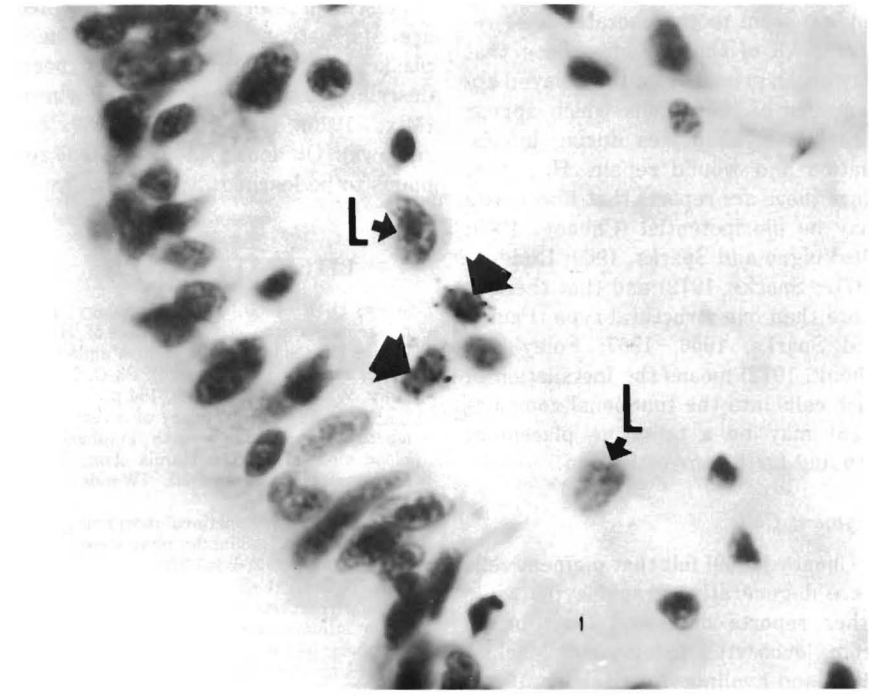


Figure 4.—Normal *O. lurida* hyalinocytes (arrows) labeled with tritiated thymidine 24 h after injection. L = leucoblasts (see Fig. 1).

become too diluted after two or three divisions, and if the bivalve has been stressed, hyalinocytes may enter the M compartment without dividing; such a mechanism is well known in vertebrate leucopoiesis. The only other candidate for inclusion in the P compartment may be the fibroblast (Cheney, 1969). However, there is very little evidence available at this time to support such a view.

Maturation Compartment

In any cell series, an almost infinite gradation of cells exists between the most immature blast cell and the mature definitive forms. These intermediate types have undoubtedly been responsible for a considerable amount of disconcertion in evaluating molluscan leucocyte structure. Thus, slightly granular hyalinocytes (Cheng and Rifkin, 1970; Foley and Cheng, 1972), cells with morphological gradations between hyalinocytes, fibroblasts, and myoblasts (Ruddell, 1971c), immature basophils (Ruddell, 1971b), basophilic granular hyalinocytes (Cheng and Rifkin, 1970) and pigment cells "forming in leucocytes" (Stein and Mackin, 1955) logically seem to constitute cells in the M compartment. An example of the confusion caused by intermediate cells is illustrated by a report (Ruddell, 1971c) that *Crassostrea gigas* hyalinocytes were observed phagocytosing various materials during wound repair. This statement has not gone unchallenged (Foley and Cheng, 1972) since results from other earlier studies had led to the conclusion that only granulocytes possess the ability to function as phagocytes (e.g., Galtsoff, 1964; Cheng and Rifkin, 1970; Sparks, 1972). A possible explanation is that since the "phagocytosing hyalinocytes" were responding to trauma, they were able to phagocytose materials prior to completing differentiation to a granulocyte. Reports of immature cells having functional ability prior to completing differentiation are not uncommon in carefully studied vertebrate CRS. There is also ample evidence that mammalian hematopoietic cells not normally associated with phagocytosis (e.g., lymphocytes) can, given the proper stimulus, acquire this capability (Miale, 1972). In fact, phagocytosis is said by some to be a potential of all vertebrate reticulo-endothelial cells regardless of their fate (Miale, 1972). Cheney (1969) reported that early maturing forms in the Manila clam possessed phagocytic capability and concluded that the rates of development in maturing and functional phases were dependent on the intensity and type of stimulus. Thus, since in both these studies (Cheney, 1969; Ruddell, 1971c) the leucocytes were responding to insults (wounds and burns), it seems likely that, given a sufficient stimulus, differentiating leucocytes may become functional before they are fully mature.

Functional Compartment

Granulocytes

Virtually all studies on bivalve leucocytes indicate that granulocytes are completely differentiated, functional cells with a finite life span; such cells in carefully studied CRS are not capable of dividing. However, Cheng and Rifkin (1970) concluded, after surveying the older literature, that mature molluscan leucocytes are capable of dividing. Not only is this inconsistent with what is known about CRS, but studies in which ^{3}H-TdR has been employed (Cheney, 1969; Tomasovic, 1973; Mix, 1975a; Mix and Tomasovic, unpubl. report) show rather conclusively that division of mature granulocytes does not occur. Labeled granulocytes are not found until several days or weeks after ^{3}H-TdR injection which strongly suggests that they are formed via differentiation pathways of other cell types and not by mitosis of preexisting granulocytes.

Foley and Cheng (1972) described *C. virginica* granulocytes that contained basophilic, acidophilic and/or refractile granules. From this observation, they concluded that the three types of granulocytes represented different stages in the life span of one cell type. There seems to be little evidence to support this hypothesis. It seems more likely that there is more than one terminal type of granulocyte for the reasons outlined below. Ruddell (1971a) had ascribed rather precise functions to basophilic and acidophilic granulocytes in *C. gigas* which suggests that these are fully differentiated cells. Both immature acidophils (Cheney, 1969) and basophils (Ruddell, 1971a, b, c) have been described in bivalves. Finally, when one considers the maturation sequence of vertebrate granulocytes, there is no a priori reason to believe that mixtures of granules are indicative of different developmental stages of a single cell type. When cytoplasmic granules first appear in vertebrate granulocytes, they are few, coarse, and wine red. The number gradually increases and the granules differentiate into three types and in the mature cells, one of these types will predominate: Acidophilic (eosinophils), basophilic (basophils), or in neutrophils, both granules are present (Miale, 1972). Clearly, additional studies are required to resolve the question of granulocyte development.

Fibroblasts

Much remains to be learned about molluscan fibroblasts which are normally associated with inflammation and wound repair (Pauley and Sparks, 1966, 1967; DesVoigne and Sparks, 1969; Cheng and Rifkin, 1970; Cheney, 1971; Ruddell, 1971c; Sparks, 1972). Sparks (1972) has reviewed the structure and function of this cell.

There are several reports of hyalinocytes differentiating or maturing into fibroblasts (Pauley and Sparks, 1966, 1967; Rifkin and Cheng, 1968; Des Voigne and Sparks, 1969; Cheng and Rifkin, 1970; Ruddell, 1971a, c; Sparks, 1972) at various times after inflammatory responses are initiated and the autoradiographic studies (Cheney, 1969; Mix and Tomasovic, unpubl. report) seem to corroborate these reports. All of these studies base that conclusion primarily on the delayed appearance of these cells which appear after the hyalinocytes during inflammation and wound repair. However, since there are reports that fibroblasts may be pluripotential (Cheney, 1969; DesVoigne and Sparks, 1969; Ruddell, 1971c; Sparks, 1972) and that there is more than one structural type (Pauley and Sparks, 1966, 1967; Foley and Cheng, 1972) means the installation of such cells into the functional compartment may be a tentative placement pending further investigation.

Pigment Cells

Cheney (1969) felt that pigment cells were degenerative granulocytes while other reports described their origin from leucocytes (Stein and Mackin, 1955) and hyalinocytes (Haigler, 1964). Ruddell and Wellings (1971) felt that pigment cells of *C. gigas* were possibly involved in the transport and processing of biological fluids which would indicate that they belong in the F compartment.

Finally, as in all CRS, leucocytes are lost from the F compartment by various routes including senesence, diapedesis, or defense reactions ("death in the line of duty" [Cronkite et al., 1959]).

The proposed leucocyte CRS model is the result of attempts to draw together information from studies that have been directly or indirectly involved with leucocyte renewal. It seems likely that the above model will be completely accurate in some respects and perhaps totally inaccurate in others. There are myriad unanswered questions which do not require listing here since most will occur to the knowledgeable reader. Nevertheless, the ultimate function of any model is to stimulate further inquiry and hopefully, this model will serve that purpose. An understanding of the kinetics of leucocyte proliferation is obviously a prerequisite to discerning not only the normal steady state renewal but also for interpreting the modalities of abnormal proliferation associated with diseases and responses to different environmental insults. Our nescience on this subject precludes understanding molluscan proliferative disorders which are suggestive of sarcomatoid neoplasia; such abnormalities have been described in this volume and elsewhere (Mix, 1975a, b; Pauley, 1969 for reviews). Obviously, a great deal remains to be learned.

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