Comparative Hematology

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Dedicated to
PROFESSOR HARVEY ERNEST JORDAN
who has made so many and such
fundamental contributions in
the field of Comparative Hematology

PREFACE

In all fields of human knowledge there are times when it seems that a "summing up" is desirable. The criteria for determining the proper time are not well defined. In the area of Comparative Hematology a short summation has been given within recent years by Knoll ('57; see Chapter 5). To find any comprehensive summary, however, one must go back all the way to Jordan, who in *Downey's Handbook of Hematology* ('38) made use of a very large amount of the earlier literature and of his extensive personal investigations to compare the blood and blood-forming tissues of a great many kinds of animals, both vertebrate an invertebrate. Because of the rapid progress in technological capabilities, this takes us to a time before any hematologic studies had been undertaken either with phase or electron microscopy.

The comparative aspects of histology had been of much interest to us for many years. One of the finest rewards from the publication of the *Textbook of Histology* (Oxford University Press, 1959) was the welcome invitation from the present publishers to prepare a volume on Comparative Hematology. While we have realized only too well the difficulty of such an undertaking, we have felt the burden lightened and its pleasure increased by the many workers in this field, both in this country and abroad, who have been of the greatest assistance in the furnishing of illustrations, specimens and, most of all, encouragement.

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Chapter 1 WANDERING CELLS IN ANIMALS WITHOUT CIRCULATING BLOOD

Porifera

In animals in which no circulatory system is present, the wandering cells generally carry out a number of functions, which include to some extent those which are served by blood cells in the animals with a circulation.

In the sponges, vast numbers of amebocytes wander through the gelatinous, transparent matrix of the mesoglea which fills in the spaces between ectoderm and endoderm. There is a considerable variety in the appearance of such cells (fig. 1-1, 1-2). Some show slender, branching pseudopods. In others the pseudopods are lobose. The cytoplasm may contain granules, pigment bodies or spherules. Some of the inclusions probably are nutritive material, others excretory substances. However, it is probable that many amebocytes are versatile and that the varied appearances seen represent activities at the time of fixation.

Particularly interesting is the activity of sponge amebocytes called scleroblasts. These cells contain the spicules which are placed together to form the skeleton (fig. 1-3). Such tiny skeletal elements, varying in shape, are composed, according to the type of sponge, of salts of calcium, or silicon, or of other materials. The scleroblasts apparently secrete the spicules, often working in groups, transport them to the proper place, and deposit them—not unlike a troop of ants!

Also important among the amebocytes of sponges are the archeocytes, or primitive cells. These are characterized by a large nucleus, usually with a conspicuous nucleolus, and relatively scanty cytoplasm with blunt pseudopods. This description we shall find later to fit very well indeed with that type of cell most widely accepted as a "stem cell" for many, if not all, types of blood cells in various kinds of animals—the so-called hemocytoblast. In the sponges, such cells are believed to represent undifferentiated cells persisting from the embryo into the adult, to be the sole source of the gametes, and to differentiate into other cell types during regeneration.

Many of the amebocytes of the Porifera are phagocytic. They receive nutritive material from the endodermal cells and carry it to other cells, such as the developing gametes.

Coelenterata

In the coelenterates, certain characteristic free cells are found, the interstitial cells. They lie in the mesoglea and among the epithelial cells, usually near their bases. They are undifferentiated and give rise to the sex cells and the defensive cells, the cnidoblasts. They

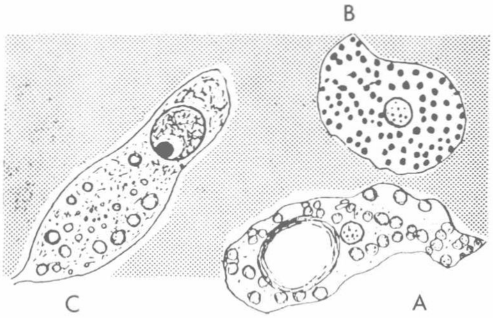

FIG. 1-1. Several types of amebocytes from the sponge, Microciona: A, globoferous cell, also called a "cystencyte"; B, granular cell or granulocyte; C, nucleolate cell, considered a more primitive type of amebocyte. (After Wilson and Penney, '30.)

take part in regenerative processes. They are comparable in many ways to the undifferentiated mesenchymal cells of vertebrates, although they resemble the lymphocytes in being "free" rather than fixed cells.

The interstitial cells are relatively small, rounded cells and arise in early development, apparently originating by division from both ectodermal and endodermal cells. They have been compared to the archaeocytes of the sponges.

Studied with the electron microscope (fig. 1-6), the interstitial cell shows a finely granular cytoplasm with no channels of endoplasmic reticulum. Small mitochondria are present, also a Golgi apparatus. The nucleus is evenly granular and shows one or more prominent nucleoli. There often appear to be cytoplasmic bridges between or among interstitial cells, forming at least small syncytia.

In places where some endoplasmic reticulum is seen in an interstitial cell, it is presumed that it already has begun to differentiate into a cell of different type, such as a cnidoblast, which does possess a prominent ER.

Ctenophora

There appears to be little known about wandering cells in the ctenophores. The collenchyme, or general connective tissue, is described as an ectomesoderm. It consists of a gelatinous matrix with numerous muscle cells, connective tissue fibers, and fixed and wandering cells in its meshes. The wandering cells are called amebocytes by Hyman ('40) who presents some drawn very roughly "from life."

Platyhelminthes

Prenant ('22), describing the parenchyma of flatworms, says that a "glance" at the hemocytoblasts of vertebrates will show how similar they are to the primitive or stem cells of

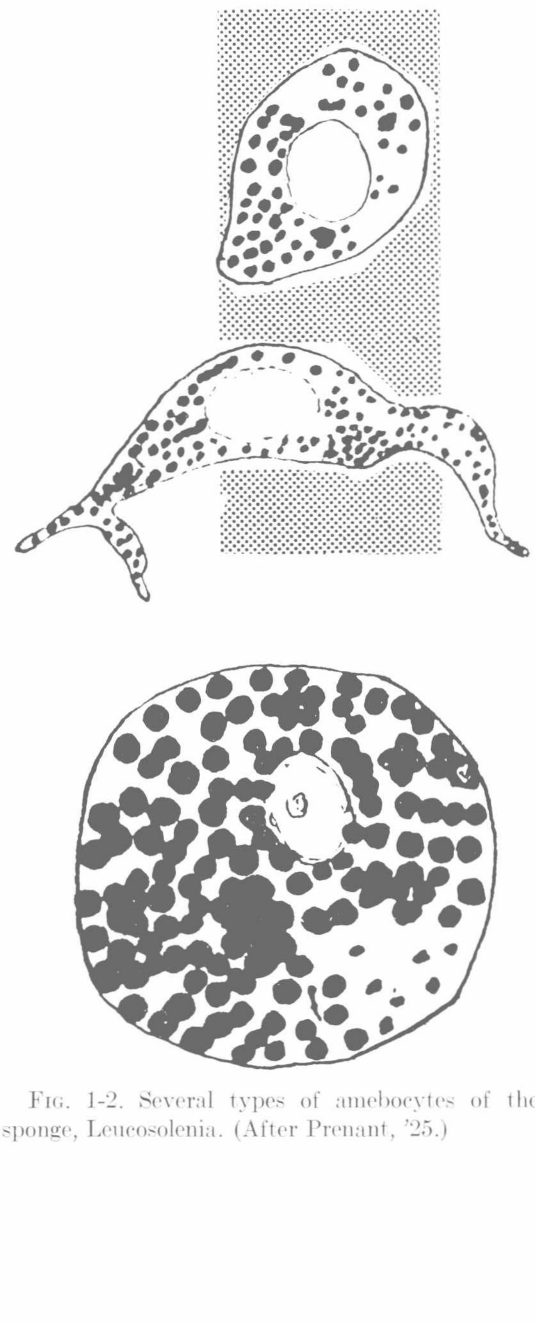

FIG. 1-2. Several types of amebocytes of the sponge, Leucosolenia. (After Prenant, '25.)

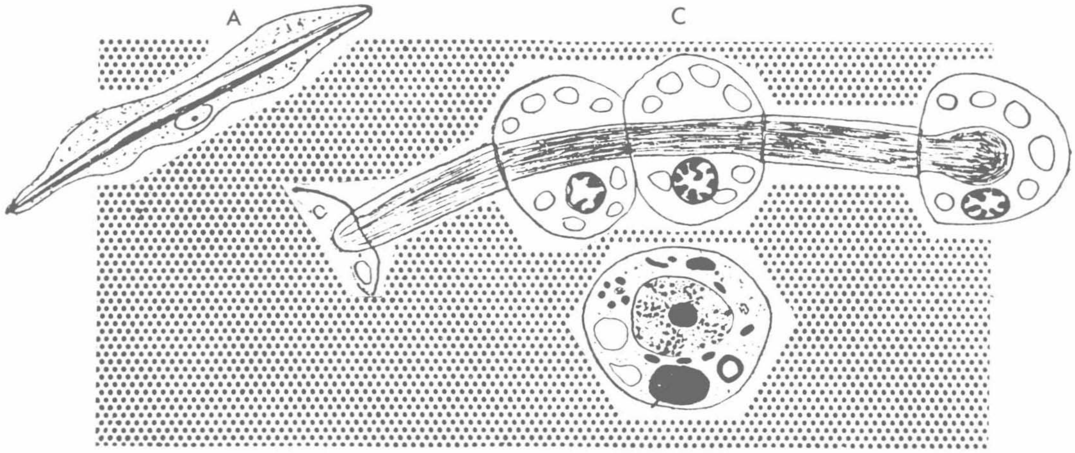

FIG. 1-3. Scleroblasts of the sponges. These are amebocytes which form the skeletal elements and carry them to the sites where they are deposited: A, cell with a siliceous monaxonal element, from a freshwater sponge, *Ephydatia blembingia* (after Evans, '01); B and C, spongioblasts of Reniera: B, with beginning mass of spongin; C, in series, secreting a spongin fiber. (After Tuzet, '32.)

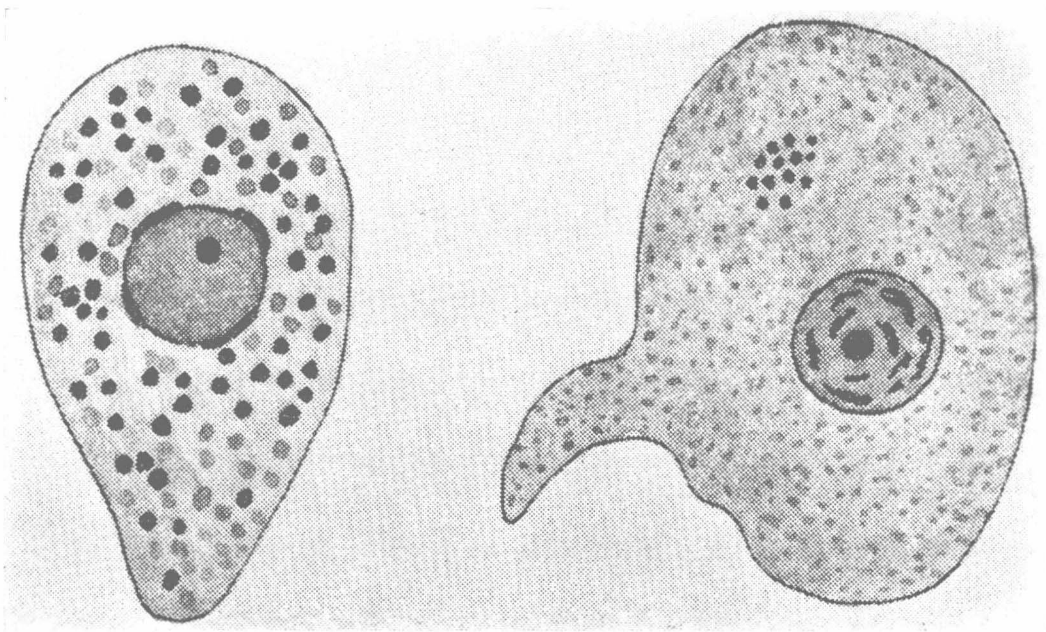

FIG. 1-4. Amebocytes of the sponge, *Sycon raphanus*, Schmidt; A, eosinophilic granulocyte; B, hyaline leukocyte. (Redrawn from Tuzet, '63.) (Courtesy of University of California Press.)

triclad and polyclad flatworms. In either case, the cell can become fixed again to form an integral part of the connective tissue. In flatworms also cells with dense nuclei, resembling the smaller lymphocytes, can develop from the hemocytoblast. The stem cells of flatworms, however, have wider potentialities than those of hemopoietic tissue of higher

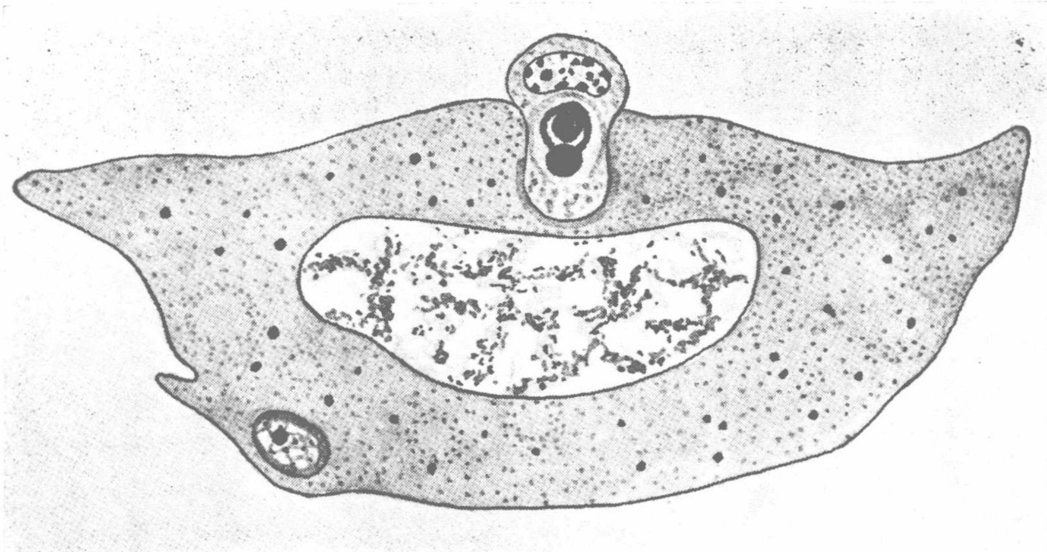

FIG. 1-5. A carrier amebocyte in the act of transmitting a spermiocyst to an oocyte in the sponge, *Grantia compressa pennigera Haeckel*. The oocyte has formed a cytostome for the reception of the wandering cell. (Redrawn from Tuzet, '63.) (Courtesy of University of California Press.)

forms, for they apparently are able to give rise even to new germ cells.

Ivanow ('52) (see figures 1-7 and 1-8) has described ameboid phagocytes of the parenchyma in the Turbellaria Acoela. They apparently function much as do the phagocytic amebocytes in sponges. While undifferentiated cells form characteristic elements in the parenchyma of this group, there appear to be at least two different kinds of phagocytes and several types of connective tissue cells.

A number of members of the class Trematoda show, in addition to the expected presence of wandering cells, a system of mesenchymal vessels which seems to represent a primitive circulatory system. It consists of four longitudinal tubes on each side, ending blindly anteriorly and posteriorly and giving off blind branches to the intestines, to the reproductive organs and into the suckers. These tubes, which may be thought of as lymph channels, are lined by flattened cells. The channels apparently function in distribution of respiratory gases and food and carrying away of excretory products. In the fluid within them are mesenchymal cells which apparently have entered through their walls. They resemble the most primitive vertebrate blood cells, the hemocytoblasts, (Jordan and Reynolds, '33). There seems to be even a center of formation of such cells at the division of the crura of the intestine. Within the mesenchymal tissue a type of eosinophilic granulocyte is seen which apparently develops from the hemocytoblast.

The cestodes do not show such a system.

Aschelminthes

This phylum now includes the Rotifera, the Gastrotricha, the Nematoda and the Nematomorpha.

The rotifers show branched ameboid cells in the fluid-filled cavity of the pseudocoel. They appear to have phagocytic and perhaps excretory functions.

In the Gastrotricha no free ameboid cells are found in the pseudocoel.

The nematodes show either a syncytial or a cellular arrangement in the pseudocoel. There seem to be no ameboid cells in relation to this connective tissue nor any cells floating free

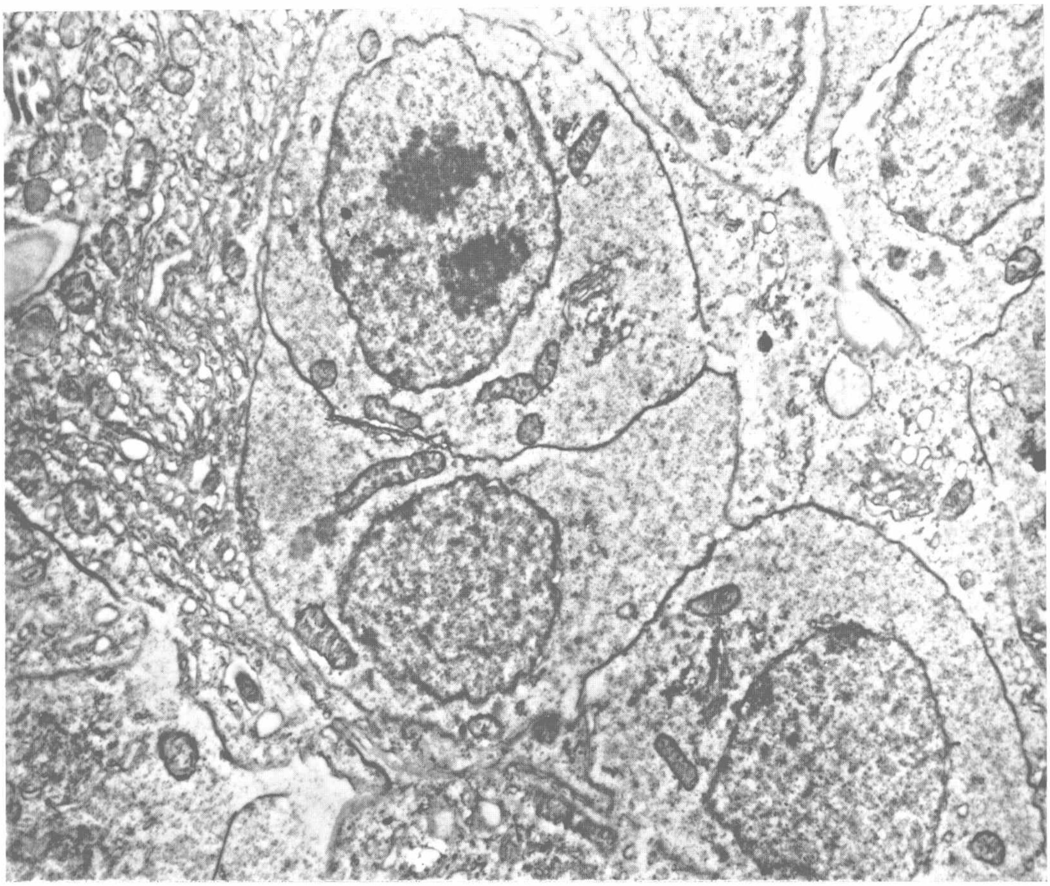

FIG. 1-6. Three interstitial cells of *Hydra oligactis*. This figure illustrates the tendency for such cells to occur in small groups. Prominent nucleoli, as in the uppermost cell, are common, and the cytoplasm, as compared with that of the adjacent epithelial cells, is relatively homogeneous. ×6500 (Courtesy of Doctor Arthur Hess.)

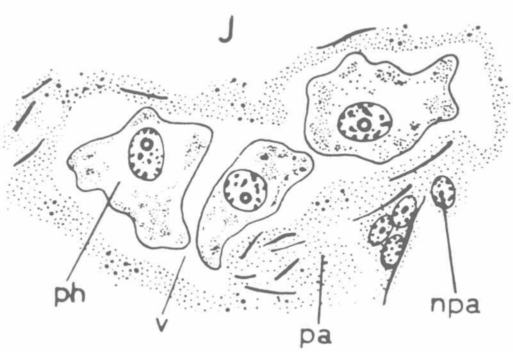

FIG. 1-7. Ameboid phagocytes in the parenchyma of the flatworm, *Oxyposthia praedator*. npa, nuclei of the syncytial parenchyma; pa, matrix of parenchyma; ph, phagocytic cells; v, vacuole in which phagocytic cells occur. (Redrawn from Ivanow, '52.) (From Ax, P., 1963. Relationships and Phylogeny of the Turbellaria, pp. 191–224.) (Courtesy of University of California Press.)

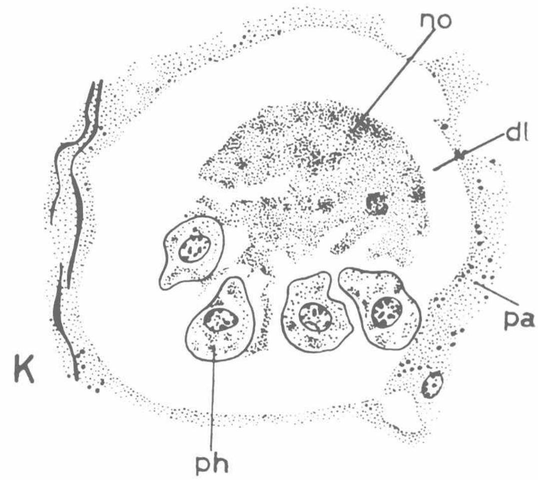

FIG. 1-8. Phagocytic cells in process of digestion in the parenchyma of the flatworm, *Oxyposthia praedator*. dl, "digestive lumen"; no, nutritive material; pa, matrix of parenchyma; ph, phagocytic cell. (Redrawn from Ivanow, '52.) (From Ax, P., see legend, fig. 1-7.) (Courtesy of University of California Press.)

in the fluid of the pseudocoel. The fixed cells are not phagocytic (Hurlaux, '42).

The Nematomorpha have a pseudocoel of varying extent. Often it is more or less filled by mesenchymal tissue. Usually, however, the alimentary canal is surrounded by a space along most of its course. We have not found descriptions of ameboid cells in these worms.

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Chapter 2 BLOOD OF INVERTEBRATES OTHER THAN ARTHROPODS

Rhynchocoela

In the nemertine worms the structure of the mesenchyme appears similar to that of flatworms, a ground substance with fixed cells among which wander free cells (Prenant, '22). The free cells here also are active in regeneration and gonad formation. In the mesenchyme are seen cells similar to the "lymphocytes" which are found in the blood.

The nemertines are the lowest forms which show a system which can be described as blood-vascular, a closed system of vessels in which a circulation occurs. The larger contractile vessels have specialized layers in their walls, including considerable muscle tissue.

The fluid in these vessels, the first *blood*, may be red, orange, yellow, green, or colorless. The fluid part, or plasma, is colorless; while the cells are rounded or oval, somewhat flattened structures, most of which seem capable of only slight change in shape. The red pigment in such cells shows the characteristics of hemoglobin (Lankester, 1872).

There are also white cells or leukocytes in this blood. In fact, they present a rather surprising variety. Ohuye ('42) described hyaline amebocytes, amebocytes with coarse or fine eosinophilic granules, amebocytes with basophilic granules, and spindle-shaped cells.

Mollusca

All members of the phylum Mollusca possess a circulatory system, with a propulsive organ or "heart" and a more or less well developed system of channels through which the blood makes its way. There is, however, a great difference in degree of development of the vessels in different kinds of molluscs. In the most primitive group, the Amphineura, represented by such animals as Chiton, the circulatory system is almost entirely a lacunar one and the blood is forced out freely between the organs.

In the Gastropoda and Pelecypoda the vessels are better developed, having definitive walls and with less of the system being of a lacunar type. In the Cephalopoda the circulatory system is the most highly developed, with definite arteries and veins and even a genuine capillary system connecting the two. This degree of development correlates with the active life of the cephalopods and the high differentiation of their sense organs. Thus it is possible in the molluscs, with the exception of the Amphineura, to distinguish the blood from the fluid of the body cavity and from tissue fluid.

The amount of oxygen in the blood varies considerably in different molluscs. The differences seem to be related directly to the characteristics and the amount of the protein which is present in the plasma. On this basis, the molluscs can be separated into two categories:

(1) Those in which the blood contains a respiratory protein, either hemocyanin or hemoglobin. Here the concentration of oxygen is greater than would be the case according to the simple laws of solution. Also, it varies according to the amount of the respiratory protein.

(2) Those in which no specific respiratory protein is present. Here the oxygen concen-

tration follows the laws of solubility in a salt solution and varies little.

The common respiratory protein of the molluscs is the blue hemocyanin. This is indeed a very important protein for it is found not only in many molluscs but also widely distributed in the great phylum of the Arthropoda. The metal forming a part of the molecule of hemocyanin is copper, in contrast to the iron component of hemoglobin. It will be recalled that solutions of copper compounds, as for example, copper sulfate, are of a blue color.

Hemocyanin is present not in the cells but in the plasma. Frederiqu (1878) was the first to describe the formation of oxyhemocyanin and to compare this compound, in which oxygen is loosely bound to the hemocyanin molecule, to the oxyhemoglobin of the red blood corpuscles of vertebrates. He obtained hemocyanin from the blood of Octopus by a process of dialysis.

Henze ('01) first obtained crystallized hemocyanin, again using the blood of cephalopods. Crystals have been obtained from a number of molluscs, including Octopus vulgaris and macropus, Helix pomatia, Sepia officinalis and Eledone moschata and from some crustaceans, such as Palinurus vulgaris. In crystallized hemocyanin, the percentage of copper (0.34–0.38 per cent) appears to be rather constant.

The blood of a few species of molluscs contains hemoglobin. It is found in some species of Amphineura, in only one genus of gastropods, *Planorbis*, and in a few species of lamellibranchs among the pelecypods. In Planorbis it is dissolved in the plasma. In the lamellibranchs it is found in the plasma in some species, while in others it is within certain cells of the blood. In the Amphineura it is present in the plasma in some types, in cells in other types.

While there is room for question as to whether *all* of the blood which is *red* in different species of molluscs contains a compound chemically identical with the hemoglobin of the vertebrate blood, it was shown at a very early date that the absorption spectrum in some cases, at least, is almost identical with that of vertebrate hemoglobin (Lankester, 1869, 1871, 1872). Others confirmed the spectroscopic similarity while Krukenberg and Mays (1880) and later Velichi ('00) obtained characteristic crystals of hemin from the blood of Planorbis. Dhéré (1903) showed the high content of iron in this blood.

Red or yellowish-red blood was found in nine different species of lamellibranchs by Griesbach (1891). In all these cases it was within certain cells. Spectroscopic and crystallization phenomena also supported the nature of the pigment as hemoglobin in the blood of these animals.

If we ask the question why certain molluscs have hemoglobin in the blood while others do not, one soon finds that apparently closely related species of the same genus may differ in this respect. One may look naturally to habitat and way of life as a possible explanation for differences in occurrence of hemoglobin, with the idea, which some have had, that in cases where absorption of oxygen has become difficult, hemoglobin is found. However, the power of absorption seems to be as great in some species with hemocyanin as in those with hemoglobin.

The colorless proteins of the blood in various molluscs have been suggested as respiratory proteins. If this were true, it would be of considerable importance, partly as indicating that a chromoprotein is not necessary to carry the oxygen, but definite evidence of such a rôle by colorless protein is lacking.

Other organic compounds present in the blood of molluscs include nitrogenous substances, sugar and, in formed elements, such as lymphocytes, droplets of fat.

The electrolytes vary in amount considerably and are in greater concentration in the marine forms. Beside the metallic ions which may be Cu^{++}, Fe^{+++}, or Mn^{++}, the cations Na, K, Ca, and Mg and the anions, Cl, PO_4, SO_4 and CO_3 are generally found in the blood of all molluscs.

Cells of the Blood

The amebocytes of mollusc blood can arise in either of two ways: (1) by reproduction of freely circulating young cells in the blood, generally by mitotic but apparently also by amitotic division; (2) by development in a truly hemopoietic organ, or "lymphocytogenous" gland in which the cells divide and differentiate somewhat as in the hemocytopoietic organs of vertebrates and from which they are liberated to enter the circulating stream. The question as to which manner of origin occurs is primarily a taxonomic one.

The blood cells possess retractile pseudopodia of varying size and form. Conspicuous in mollusc blood cells are the numerous strongly refractile granules (fig. 2-1, 2-2). In some cases these granules are colored (color plate III). The nucleus may be centrally or eccentrically located. Usually it is rounded but not infrequently it is lobed or oddly shaped, a feature seen frequently in granulocytes of vertebrates. Often the granules are so abundant as to obscure the nucleus, but its presence generally can be brought out with the use of proper reagents.

In relation to staining properties, the granules in the majority of cases are acidophilic. In smaller numbers, cells with basophilic or with neutrophilic granules are seen. While various attempts have been made to classify the different kinds of granular leukocytes in molluscs, it is not yet certain whether the possible categories actually represent different stages in a developmental series or in several such series.

Conspicuous among the functions of blood cells in molluscs is that of excretion. Particularly in the lamellibranchs, wandering cells laden with inclusions may be seen making their way through the tissues.

It has been suggested that the amebocytes take part in storing up of reserve food material, fats and proteins for the tissue cells and that they wander into the tissues to transport such substances (Cuénot, 1891). Whether the granules in some cases actually represent the transported materials, or whether they are the source of enzymes aiding in assimilation and transfer, is not clear.

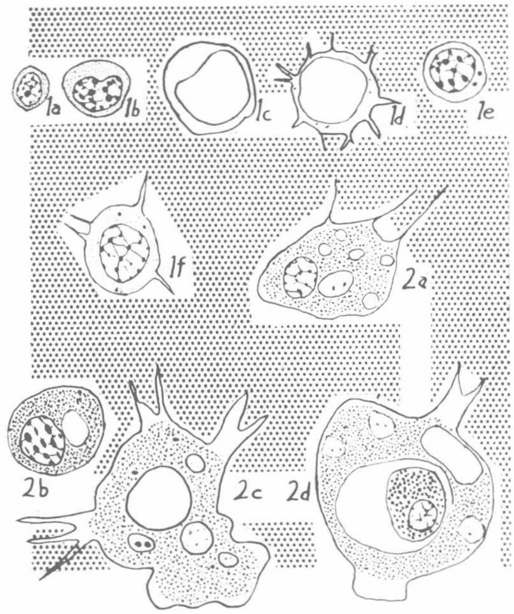

Fig. 2-1. Cells from the blood of the gastropod mollusc Busycon. Lymphocytes and macrophages, with a probable transitional form (1f) are shown: 1a-e, lymphoid cells; 2a-d, macrophages. (Redrawn from George and Ferguson, '50.)

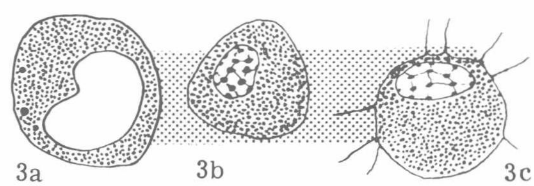

Fig. 2-2. Cells from the blood of the gastropod mollusc Busycon. These are described as eosinophils. Like the eosinophils of vertebrates, they seem to be non-phagocytic. (Redrawn from George and Ferguson, '50.)

The cells of mollusc blood often are very actively phagocytic for foreign bodies, including microbes.

In addition, the special blood cells which contain chromoproteins, as in a number of the lamellibranchs, are respiratory in function and analogous, at least, to the red corpuscles of the vertebrates.

The lymphocytes, or non-granular blood cells, are active in the clotting process. They