nearly all of heparin's anticoagulant activity measured by APTT was lost (Fig. 3B); the WBRT test gave identical results. The azure assay again demonstrated direct loss of heparin. Although it might be expected that removal of heparin would cause clotting, blood continued to flow unrestricted through the filter even after six passes. Blood samples were also taken at sample port a. After one pass there was an 80 percent loss of heparin by APTT (70 percent by azure) and after two passes there was a 90 percent loss by both assays (Fig. 3B). Heparin levels measured at sample port a were consistently higher than those measured at sample port b, presumably due to the mixing of blood in the dog. Control reactors without heparinase had no detectable effect on blood heparin levels in vivo (an in vivo half-life of 2 hours for heparin at this dosage was measured in the dog). The dogs still appeared healthy 3 months after the experiments. Blood was taken from the dogs 1 and 2 months after the experiments, and no antibodies to heparinase were detected by Ouchterlony precipitin tests (*18*). Products of the enzymatically degraded heparin were tested for cytotoxic and mutagenic effects on *Salmonella typhimurium* (*19*). No toxicity or mutagenicity was observed, even with concentrations 1000 times in excess of those we would anticipate clinically. In addition, heparin was neither toxic nor mutagenic by this assay.

At the end of the experiment, blood taken from the dogs showed no decrease in hematocrit, a 30 percent decrease in white blood cell count, and a 70 percent decrease in platelet count. These values are typical of those obtained for tests of extracorporeal circuits in dogs (*1, 20*). To further improve the blood compatibility of immobilized heparinase, other heparinase supports and the use of a smaller filter should be examined. The latter is possible because heparinase has been purified to specific activities 70 times greater (with low recovery) than the enzyme used in this study by phosphocellulose ion-exchange chromatography and isoelectric focusing (*9*).

An immobilized heparinase filter could be used either at the end of a clinical procedure to eliminate heparin without the toxic effects of heparin-neutralizing substances such as protamine or continuously to prevent high levels of heparin from ever entering the patient. Blood filters as large as 2 liters are often used at the effluent of extracorporeal devices to remove microemboli. Heparinase might be bonded to the biomaterials of these filters. Unlike many proposed medical applications of immobilized enzymes such as asparaginase (*21*) and phenylalanine ammonia-lyase (*22*), immobilized heparinase would be used in procedures in which blood must enter extracorporeal circulation and in which existing biomaterials already interact with blood at the desired location (the end of the extracorporeal circuit). Thus this process may not require any additional apparatus or invasive procedure.

The heparinase filter may also be a prototype for other selective drug removal systems. Microbial selection techniques (*8*) could produce enzymes for use in filters to degrade potentially toxic drugs such as amphetamines or barbiturates. Such drug removal systems, like drug delivery systems (*23*), could prove useful in controlling drug concentrations in the blood.

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Presence of Coelomocytes in the Primitive Chordate Amphioxus (*Branchiostoma lanceolatum*)

Abstract. *Previously undescribed free and fixed cells in the coelomic cavity of* Branchiostoma lanceolatum *respond immunologically to particulate antigens. This species is thus an important model for future investigations of the phylogeny of the immune system.*

In an attempt to explain the phylogenetic origins of the immune system in vertebrates, comparative immunologists have been studying the immune responses of primitive chordates and other deuterostomes (*1–8*). Urochordates (*9*) and echinoderms (*10*) have received much attention. However, the cephalochordate *Branchiostoma lanceolatum*, which is often regarded as sharing a common ancestry with the vertebrates (*11–13*), has been the subject of few immunological investigations (*14, 15*). Indeed, but for the hemagglutination work of Bretting and Renwrantz (*16*) and De Benedictis and Capalbo (*17*), the mechanisms by which this animal identifies antigens and defends itself against disease would be entirely enigmatic.

Although the structure of the vascular

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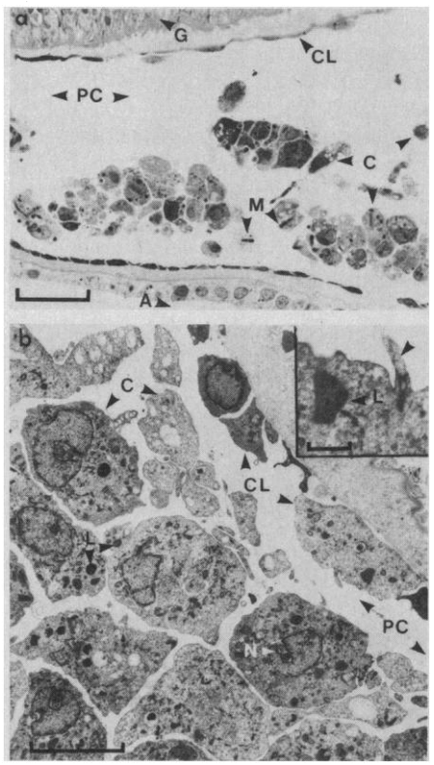
system of *Branchiostoma* is well documented (*18–26*), most authors (*2, 18, 19, 22, 23, 27*), with the exception of Hilton (*20*), Rohon (*25*), and Welsch (*26*), have reported an absence of free or fixed blood cells in this animal. The possible existence of analogous counterparts to blood cells in the coelom (coelomocytes) has also not been determined (*9*). The reported lack of free or fixed leukocytes is perplexing, as most invertebrates possess such cells (*28*). The small size and frequent unavailability of this cephalochordate and the failure to prepare horizontal sections in epoxy resin have undoubtedly contributed to the conflicting information regarding free or fixed "blood" cells. We now report unequivocally the existence of coelomocytes in *Branchiostoma* and the fact that these and the cells lining the coelomic cavity readily phagocytose bacteria.

Horizontal sections of adult *Branchiostoma* showed the presence of small numbers of free cells in the perivisceral coelom (Fig. 1a). These cells typically possessed a cleft nucleus, lysosome-like bodies (Fig. 1b), and often, cilia and rootlet structures (Fig. 2a). The cells lining the perivisceral coelom displayed no discrete lateral junctions (Fig. 2b) and also contained lysosome-like bodies, cilia, and rootlet structures (inset in Fig. 1b). These observations indicate that coelomocytes may originate from the coelomic lining, as reported in some echinoderms (*10, 29*), although distinct leukopoietic sites may also exist, as reported in certain tunicates (*9*).

To determine the functional significance of these coelomocytes, we injected bacteria into the perivisceral coelom. Both the free and the lining cells were capable of phagocytosing the test particles. The coelomocytes, in contrast to lining cells, often ingested large numbers of bacteria. The debris and myelin whorls sometimes observed in these free cells may represent previously phagocytosed and degraded material. It is not known whether *Branchiostoma*, like many other invertebrates (*28*), relies on serum-independent phagocytosis as a means of internal defense or whether the coelomocytes are part of a more specific and complex immune system. The cells lining the coelom may represent a primitive reticuloendothelial system.

Branchiostoma coelomocytes show little structural similarity to the specialized leukocytes of the urochordates, and they are less well differentiated than the phagocytic granular leukocytes of this group (*9*). They do resemble the phagocytic amoebocytes of echinoderms, although *Branchiostoma* coelomocytes possess no petaloid cytoplasmic extensions (*10*). As in *Branchiostoma*, the perivisceral coelom of echinoderms is usually lined with a ciliated endothelium (*10*). Kaneshiro and Karp (*30*) recently observed echinoderm coelomocytes containing basal bodies and striated rootlets, although, except in *Arbacia punctulata* (*31*), these structures are not typically associated with phagocytic cells. Echinoderm amoebocytes possess a relatively sophisticated recognition system capable of discriminating between closely related proteins and cells (*10, 32*). These amoebocytes and the coelomocytes of *Branchiostoma* demand further investigation, since separate cell lines with different functions may have evolved from such ancestral forms, resulting in the specialized leukocytes of vertebrates.

In conclusion, *Branchiostoma* possesses free cells capable of responding to particulate antigens. The site of origin, degree of specificity, and functional modality of these cells may assist compara-



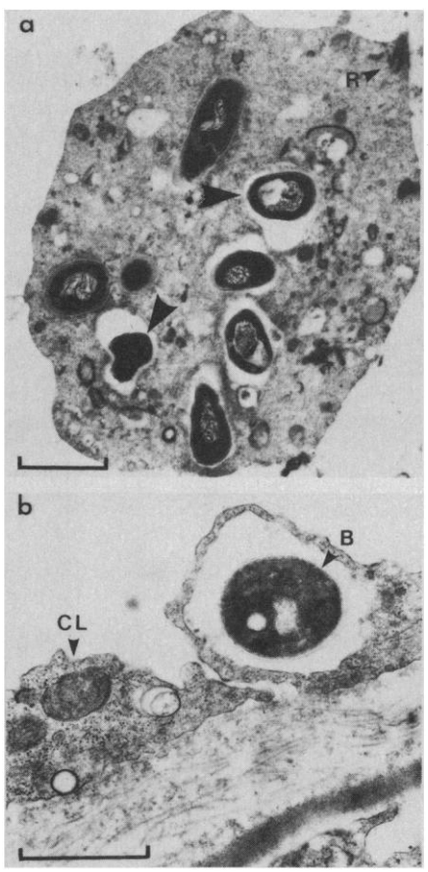


Fig. 1 (left). (a) Low-power micrograph of a section of *Branchiostoma*, showing the atrium (*A*), gut (*G*), perivisceral coelom (*PC*), coelomocytes (*C*), and endothelial lining cells (*CL*). Phagocytosed bacteria (*M*) are present in some free cells. Live *Branchiostoma* from the Marine Laboratory (Plymouth, United Kingdom) were maintained in circulating water in laboratory aquariums. Fourteen specimens were anesthetized with tricaine (0.5 mg per milliliter of seawater) and surface-sterilized. A sterile 30-gauge needle was then used to inject *Bacillus cereus* (NCTC 2599) or *Moraxella* sp. (NCMB 308) (1×10^8 bacteria) into the perivisceral coelom. The animals recovered rapidly and were returned to their aquariums for 4 or 18 hours prior to sampling. Uninjected and saline-injected specimens acted as controls. Transverse tissue slices from the gut region were fixed in 4 percent glutaraldehyde, postfixed in 1 percent osmium tetroxide, and processed with standard electron microscope techniques before being embedded in Araldite or L.R. White Resin (London Resin Company). The sample shown was removed 4 hours after injection with *Moraxella* sp., embedded in Araldite, sectioned horizontally, and stained with Mallory's azure II and methylene blue. Scale bar, 20 μm. (b) Electron micrograph of *Branchiostoma* section, showing coelomocytes in the perivisceral coelom. Note the typical cleft nucleus (*N*) and lysosome-like bodies (*L*). Endothelial lining cells are also present, some of which are ciliated and contain lysosome-like bodies. The inset shows such a body (*L*) and a cilium (unlabeled arrowhead). The sample shown was removed 4 hours after injection with *Moraxella* sp., embedded in L.R. White Resin, sectioned horizontally, and stained with aqueous uranyl acetate and lead citrate. Scale bar, 5 μm; scale bar to inset, 0.5 μm. Fig. 2 (right). Electron micrographs of *Branchiostoma* sections, showing (a) coelomocyte with rootlet structure (*R*) and phagocytosed *Moraxella* sp. (unlabeled arrowheads) and (b) endothelial lining cells (*CL*) with phagocytosed *Bacillus cereus* (*B*). The samples were processed as described in the legend to Fig. 1b. Scale bars: (a) 1.5 μm and (b) 1 μm.

tive immunologists in further understanding the phylogeny of the vertebrate immune system.

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The Retina of the Newborn Human Infant

Abstract. *We have examined a pair of eyes from a normal, full-term infant who died at 8 days as a result of accidental injury. Eyes were obtained immediately after death, fixed, and sectioned for light microscopy. Results from both eyes were substantially the same. The macular region was still drastically immature at 1 week. Even though a foveal depression existed, all cell layers were still present across it. Furthermore, the inner nuclear layer was divided into two separate layers. The receptor layer was reduced to one or two cells thick; receptors had both inner and outer segments, but they were very short and stumpy. The region of immaturity covered about 5° of the retina. These findings suggest that the central region of a human infant's retina is probably not fully functional at birth.*

The visual capacities of infants are different from those of adults; behavioral and physiological studies have shown that an infant's visual capacity, particularly acuity, improves rapidly over the first few months of life (*1*). It is important to know the extent to which these differences between the capacities of infants and adults are accounted for by changes in retinal anatomy rather than by changes in higher centers. We now describe the state of the infant retina at birth and suggest that postnatal development of the retina may account for some of the improvement in visual capacity.

Little information is available about the human infant retina at birth. Standard sources (*2*) state that, at birth, the peripheral retina resembles that of the adult, but that the macular area is still immature; across the fovea, the outer nuclear layer is very thin, inner segments of receptors are broad, and outer segments are short and stumpy. It is only by the fourth month that the foveal cones are said to reach their full slender length and that the inner nuclear and ganglion cell layers move to the side to form the adult foveal depression. Most of these conclusions about foveal immaturity seem to be derived from work done early in this century; only drawings—not photographs—are presented, and the number of infants and the reasons for their deaths are unknown (*3*).

The development of the fovea has been more fully explored in macaque monkeys, who also show considerable increase in acuity after birth (*4*). In the monkey the fovea is still immature at birth (*5*), but the immaturity is not as drastic as suggested by the human data. It is often assumed today that the earlier work on humans overstated the degree of foveal immaturity at birth (*6*). We have undertaken to resolve this issue in order to facilitate the interpretation of measures of human infant visual function during development.

We have examined the retinas from several human infants at late prenatal and early postnatal ages. One especially important pair forms the basis of this report. The eyes were obtained from a normal, full-term male infant with no congenital anomalies who died at postnatal day 8 as a result of accidental burns. The eyes were removed 1 hour after death. One whole globe was fixed in Susa fixative containing picric acid, dehydrated in alcohol, embedded in low-viscosity nitrocellulose, serially sectioned at 10 μm, and stained with a modified Cason's stain (*7*). From this eye we have a complete set of regularly spaced sections across the entire eye, which allowed unambiguous identification of the macular region. The second eye was fixed in 2 percent paraformaldehyde and 2 percent glutaraldehyde in *p*H 7.4 phosphate buffer; it was then cut into identified areas and embedded in Epon. The block including the macula was serially sectioned at 2 μm, and sections were stained with azure II–methylene blue.

The macular region was still very immature, while more peripheral regions resembled those of the adult. Figure 1 (A to C) shows a series of photomicrographs taken at different positions along a single 10-μm horizontal section which included both the optic nerve head and the fovea. In the peripheral regions all retinal layers appear well-developed and mature (Fig. 1A).

In the nasal retina immediately adjacent to the fovea, major differences from the adult retina first appear. As the fovea is approached (right side of Fig. 1B), the inner nuclear layer begins to split into two distinct layers, the number of cells in the outer nuclear layer decreases, the layer becomes thinner, and the space between the external limiting membrane and the pigment epithelium decreases. At the fovea (Fig. 1C) there is a depression in the retina which is marked by a decrease in the thickness of all nuclear layers; however, both the ganglion cell and inner nuclear layers still extend across the fovea. The split in the inner nuclear layer extends to the edge of the fovea, possibly across the fovea, and is even more marked than in Fig. 1B. The outer nuclear layer is reduced to one or two cells thick, and there seems to be little space for inner and outer segments

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