

Balanoglossus and the Origin of the Vertebrate Nephros¹

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It was in 1886, writing on "The Ancestry of the Chordates", that William Bateson (4) made the following remarks: "Upon the origin of the excretory system of vertebrates nothing can be affirmed from a study of *Balanoglossus*. The excretory systems of Vertebrates cannot be easily derivable from anything found in either *Balanoglossus*, *Ascidians*, or *Amphioxus*. The absence of any regular excretory system in these three forms may, perhaps, be correlated with the extraordinary development of their respiratory systems which may possibly assist in this function. The one fact which is derivable from the morphology of *Balanoglossus*, *Ascidians* and *Amphioxus* is that it is nearly certain that the excretory system of other Chordata has been developed within the group."

Observations made during the sixty-odd years since those words were written by no means invalidate them in their entirety. With excretory organs of one kind or another long since known for all three groups, the statement on "absence of any regular excretory system" obviously does not hold. But the question of evolutionary origin of the vertebrate nephros, implicit to the final remark, remains as much a puzzle today as it did then.

The structural unit of the vertebrate kidney is the nephron. While they exhibit progressively greater complexity in the evolutionary march from cyclostome to mammal, all nephrons are built to the same basic pattern: a tubule equipped with an elaborate blood supply. This tubule is invariably derived from coelomic epithelium, i.e., is mesodermal in origin, and may, although normally not, show its relation to the coelom by remaining open thereto. The blood supply consists of a filtering bed composed of a capillary tuft, or glomerulus, associated with the central or coelomic end of the tubule and a second set of capillaries surrounding the tubule proper. The distal ends of the multitudinous nephrons composing a kidney ultimately empty into a common collecting duct. This is the kind of excretory unit which appears abruptly and full-blown on the evolutionary scene, having been identified even in the oldest vertebrates known, the ostracoderms, and about whose invertebrate origins we should like to know more. Do present-day protochordates offer excretory parts suggestive of a kind which by some remodeling could have become vertebrate-like?

The tunicates are certainly not very helpful. They have been described as possessing clusters of vesicular cells usually associated with some division of the digestive tube. These range from a loose assemblage of cells in the more primitive forms through an agglomerated

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visceral mass to a large vesicular body in the Molgulidae, the most specialized of the ascidians. All are products of the vascular mesothelium and in the last instance the individual cells have fused to form the single bladder-like structure. The evidence that such bodies, whatever their makeup, are truly excretory organs is somewhat circumstantial. Most of the argument rests on the presence within the cells of crystalline masses of uric acid, although the ability of the vesicles to take up methylene blue *in vitro* has also been cited (Asema, 1). Even granting their excretory nature, they certainly bear no resemblance to nephrons. They thus offer just one more piece of evidence supporting the view that the tunicates are off the main line of vertebrate evolution, constituting instead a group of degenerate and specialized descendants of primitive and as yet unknown ancestral chordates or possibly even a vertebrate.

Amphioxus, although exhibiting many fundamental chordate features, has an excretory system consisting of segmentally arranged protonephridia with solenocytes such as occur in certain polychaete annelids (Goodrich, 7, 8 and as Minot and Boveri before him knew). These elements are ectodermal in origin and are otherwise only analogous to vertebrate nephrons. How Amphioxus, so vertebrate-like in many other respects, acquired excretory tubules of this sort remains a complete mystery. The mystery deepens in the light of current opinion (e.g. Gregory, 10) which, following a suggestion by Dohrn (6), looks upon Amphioxus as a degenerate derivative of a primitive vertebrate (ostracoderm?) rather than the reverse.

In terms of excretory organs alone, the placing of Amphioxus in a fundamental position as a stem form, would require a return to some kind of scheme deriving the chordates from annelids. Goodrich's proposal in this regard is interesting. He identified vertebrate nephrons with reproductively functioning coelomoducts of annelids. It is true that vertebrate excretory tubules are coelomoducts both morphologically and embryologically, and is also true that certain of them perform genital functions in male vertebrates. By linking those facts with those occasions in annelids where nephridia and genital tubes become united in a common system, one might go far in spinning the web of an argument linking annelids and vertebrates. But an entire generation of morphologists has scrutinized and argued the annelid hypothesis of chordate origin and the contradictions in other organ systems are too profound. It is rather unlikely, therefore, that the annelids as a group and Amphioxus with its as yet unexplained annelid-like excretory system have any relation to or throw any light on the origin of vertebrate nephrons.

Turning to the balanoglossids, one finds an organ, presumably excretory in nature, which has interesting implications. It is associated with that dorsal diverticulum from the digestive tract that runs some distance forward into the basal part of the proboscis. Called the stomochord by certain authors, the diverticulum has been homologized (by Bateson) with the notochord. The soundness of the homology, debated *in extenso* by a succession of morphologists, is not our concern.

Above and below the stomochord or notochord, as you will, are blood vessels. The dorsal vessel is expanded as a conspicuous sinus from which branch vessels supplying the proboscis and connecting with the ventral vessel. Of principal interest are numerous finger-like projections, or aneurysms, from the dorsal sinus which extend to either side and around the cranial end of the stomochord. The sinus and its aneurysmal derivatives are bounded by the mesothelium of the coelom. Called by Bateson the "proboscis gland", the collection of branching sinuses and associated mesothelium has been termed a "glomerulus" by Spengel (15) and others and excretory functions have been attributed to it. Even though the mesothelium is not organized in the form of tubular nephrons, the makeup of this organ is such as to provide the essential features of a vertebrate excretory organ, namely, a great area of contact between blood and coelomic epithelium. In its simplest form, such as is found in *Protobalanus*, the glomerulus consists of a few symmetrically disposed vascular projections bounded by an undifferentiated epithelium. More commonly, however, the organ is elaborately and complexly branched, the mesothelium considerably specialized, and the whole a surprisingly conspicuous body in the proboscis cavity. Descriptions of such glomeruli, some fairly complete and other superficial, have been given by numerous workers for a variety of species (van der Horst, 11 and Dawydoff, 5). I should like to add to these some observations and comments on the glomerulus of *Ptychodera bahamensis*.

Specimens used in the study were collected some years ago at Tortugas, Florida by Professor Fernandus Payne. Professor Payne kindly turned over this material (stored in alcohol following bouin fixation) to me. Sections, both transverse and sagittal, were cut at thicknesses ranging from 8 to 15 microns. Most were stained by the Azan method, but Ehrlich's hematoxylin, Acid Fuchsin, Orange G-Aniline Blue, and Iron-Hematoxylin were also employed.

The gross form of the glomerulus (Figs. 1, 2 and 3) is that of a narrow, elongate, bilobed mass. Its anteriormost portion abuts the terminal tip of the stomochord with each half lying on either side of the central blood sinus and a partition of connective tissue. Each lobe then extends backward as a tapering arm on either side of the stomochord. Anteriorly the arms of the glomerulus are dorsolateral to the stomochord, but at the level of the broader, irregular base of the chord the arms are strictly dorsal (Fig. 3). Absolute figures may not mean much, but one set of measurements made on a full-grown adult specimen 18 cm. long gives a suggestion of general proportions. The glomerulus in question had a total length of 4.6 mm. The two lobes together in front of the stomochord had a broadest diameter of 0.5 mm.; the greatest diameter of either arm was 0.37 mm.

In describing the general organization of the glomerulus, one hardly knows whether to speak of an elaborately branching and anastomosing network of blood sinuses bounded by mesothelium or an area of intricately folded, branching, blindly-ending mesothelial projections, somewhat akin to a placental villus, within whose maze blood slowly circulates. Perhaps the latter, with its emphasis on the meso-

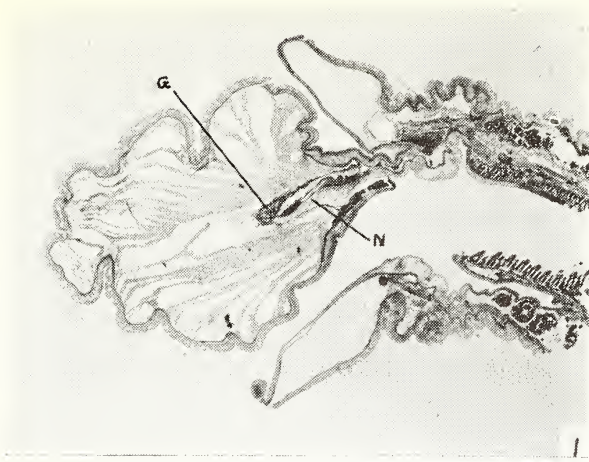


Fig. 1. Longitudinal section through collar and proboscis. x25 G,glomerulus; N,notochord.

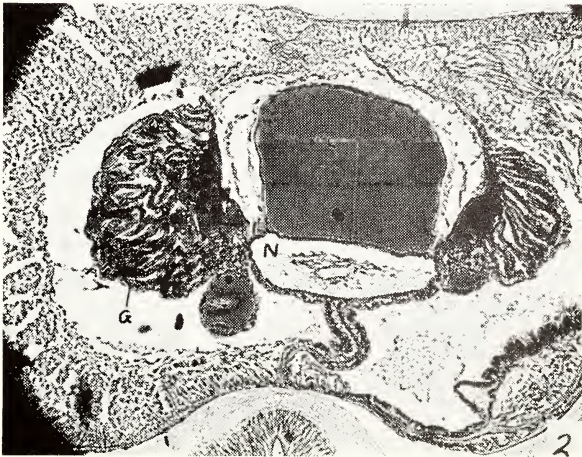
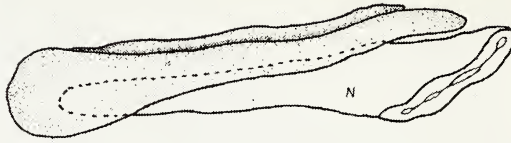
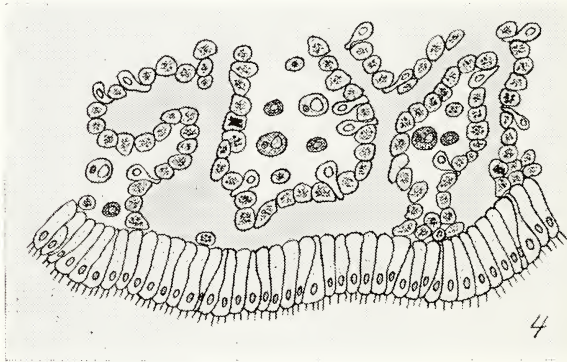


Fig. 2. Transverse section of lobes of glomerulus,G x100 N,notochord; blood sinus in center.



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Fig. 3. Diagrammatic reconstruction of glomerulus. N, notochord.



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Fig. 4. Semidiagrammatic drawing of portion of glomerulus. x900
Description in text.



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Fig. 5. Coelomduct, C, opening into first gill chamber. x100.

thelium, portrays the situation the better, for the central blood sinus and its aneurysms are completely devoid of a bounding vascular endothelium. Their walls are only those provided by the coelomic epithelium: the walls of the so-called pericardial cavity and the proboscis coelomic (Fig. 2). This arrangement of coelomic epithelium serving as a vascular wall may possibly anticipate that situation in certain vertebrates wherein the intermediate mesoderm not only provides the nephrons but furnishes cells destined to form the walls of the aorta and cardinal veins. Be that as it may, the important thing in this balanoglossid is the presence of a relatively enormous contact area between vascular liquid and coelomic epithelium. Since the role played by this association presumably is excretory, we should examine its prospects further, i.e., inquire into its capacities with respect to the two principal functions of a vertebrate-type excretory organ: **osmoregulation and excretion of nitrogenous waste.**

With regard to the former, the following comments are frankly speculative. They are offered here not as conclusions but as a tentative assessment and evaluation of ideas preliminary to any experimental approach which I, or other workers, might some time have the opportunity to make.

We have come to think of the marine invertebrates as being in osmotic equilibrium with the sea and thus facing no problem of water regulation. So it was presumably with the chordate ancestor of the vertebrates: its excretory system must have been little concerned, if at all, with water control. Only when the vertebrates ventured into fresh water were they faced with the problem of protection against excessive hydration. Smith (13, 14) has made the interesting suggestion that the armor encasing the early fishes was evolved as a protective water proofing. But this alone was not enough, for the digestive and respiratory systems still afforded routes for the absorption of excessive water; hence the origin of the glomerulus whereby the surplus water could be filtered out and eliminated via the nephrons. Concomitantly, of course, the nephrons became capable of reabsorbing glucose, salts and the like which otherwise would have been lost through the filtration system.

The return of some of the fishes to the sea and the evolution of land forms of course reversed the problem: water conservation rather than elimination became the issue and the glomerulus was a liability rather than an asset. This difficulty appears to have been solved in a number of ways. Nearly all modern marine teleosts show more or less reduction in number and size of the glomeruli, culminating in some which have become completely aglomerular (Grafflin, 9, and Marshall and Smith, 12). They also eliminate large amounts of salt through the gills. The elasmobranchs, on the contrary, employ an entirely different scheme. They have evolved a specialized segment of the nephron which reabsorbs urea and returns it to the blood where it reaches concentrations of as much as 2.0-2.5%. This raises the osmotic pressure of the blood to a level slightly higher than sea water and brings about an inflow of water. Retaining their large glomeruli, marine elasmobranchs

are in effect similar to fresh water teleosts in their technique of osmoregulation, i.e., they take in water and eliminate a hypotonic urine.

Among terrestrial vertebrates we find the problem of water conservation handled somewhat differently. The reptilian-avian line patterns itself after the marine teleosts by reducing the glomerular system, although no completely aglomerular kidney occurs. In the further interest of conservation, water is also reabsorbed via the cloaca plus, in birds, a specialization of a segment of each nephron, the loop of Henle, for water resorption. The last named scheme, tubular resorption, is employed by mammals.

The point of this excursion into the physiological phylogeny of the vertebrate nephros is simply this. If the enteropneusta are, like the marine invertebrates, in osmotic balance with their environment, then the glomerulus must have nothing to do with water control. On the other hand, if the glomerulus is truly vertebrate-like, i.e., is a water filter, then one must search for those physiological conditions tending to bring the animal into osmotic disharmony with the resultant need for such a filter.

Turning now to the other aspect of excretion, the elimination of nitrogenous wastes and other materials, one can, in the absence of physiological data, only see what the structural detail of the glomerulus, meaning the coelomic epithelium, has to offer (Fig. 4). The following description is based on iron-hematoxylin stained material.

The bounding epithelium, that is, that facing the proboscis coelom, is columnar (here and there pseudo-stratified) in type and ciliated (as is the entire coelomic lining). The oval, darkly-staining nuclei, rest distally (at the coelomic ends of the cells) and are arranged so regularly as to give the illusion of a dark stripe traversing the epithelium. The cytoplasm is light in color and rather granular.

As one follows the folds and septa of mesothelium inward, the narrow columnar cells quickly give way to shapes both cuboidal and spherical. Those which flank the blood sinuses are mostly similar in kind, with one exception to be noted. The common type is one cuboidal to round, with highly staining granular cytoplasm and rather obscure nucleus. Such cells are arranged loosely in rows which at one and the same time serve as walls to the blood sinuses and bound the numerous irregular spaces between the equally irregular sinuses. The exceptional cells are occasionally occurring flask-shaped ones (Fig. 4) whose orientation with the neck of the cell attached to the blood sinus suggests the detaching of the cell from its neighbors to become free in the nearby intersinus space.

The intersinus spaces are by no means empty, but contain, on the contrary, a considerable variety of cell forms. In addition to cells similar in size and appearance to those in the flanking walls, at least three other types have been seen (Fig. 4). One is a cell of essentially the same size as those mentioned, but with a very granular and darkly staining cytoplasm and somewhat lighter nucleus. The other two are cells with a diameter half again as large, both with a darkly staining

nucleus and a conspicuous vesicular body. They differ only in that one is somewhat more granular and darker than the other. It should be added that mitotic figures are fairly numerous throughout.

One can only speculate as to the meaning of these cell types. It may very well be they are all variations on one theme, each reflecting differences in physiological state. Furthermore, it is possible they play an excretory role somewhat as follows: cells flanking the blood sinuses pick up waste, detach themselves, and ultimately are swept out of the coelom via the coelomoducts (Fig. 5) which empty into the first gill chamber. Or again we may be dealing with developing phagocytes and an incipient reticulo-endothelial system, not uncommon in conjunction with the excretory system (Torrey, 16). One might also argue for their excretory nature by likening them to similar cells found scattered at random throughout the balanoglossid body, especially among the muscles of the proboscis, and designated nephrocytes by some workers. The one principal point of similarity is the flask-shaped outline of these nephrocytes as they are proliferated from the epithelium bounding the proboscis coelom, later to become spherical and somewhat vesicular bodies among the muscles. But since there is no real evidence that these so-called nephrocytes have an excretory function, it would be unwise to make too much of the similarity.

To sum up and conclude, the glomerulus of *Ptychodera* morphologically appears to provide all the requisites of an excretory organ of the vertebrate type, to wit, an extensive contact area between blood and coelomic epithelium. In the present state of our knowledge, however, it would be unwise to state unequivocally that it is a kidney and argue therefrom for the vertebrate relationship of the balanoglossids. First, there remains to be answered the question of the need for a water filtering device in an animal presumably in osmotic balance with its environment. Secondly, the whole fabric of the chemistry and physiology of the coelomic epithelium and the cellular derivatives thereof awaits ravelling. In the meantime, the glomerulus can at best be only a weak argument for the vertebrate affinities of the Enteropneusta.

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