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TRANSLATION

Developmental and Comparative Biological Study of Primo Vascular System

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The deceased

Translation and edition with figures by Kwang-Sup Soh

1. Developmental Study

We proposed to investigate the developmental processes of primo vessels (PV) and their differentiation processes in order to study the development of the primo vascular system (PVS). For this, we performed systematic research with chicken eggs incubated for various time intervals.

1. At time zero, scattered basophilic granules (BG) were observed between the vitelline and the ectoblast (ectoderm). In addition, there were BGs in the spaces between the cells of the germ layer.
2. Five hours incubated egg: BGs were sometimes scattered, congregated (grouped), or arranged in order.
3. Seven to eight hours: Mesoderm (mesoblast) began to differentiate. Special looking cells which were different from mesoblast cells appeared between the ectoderm and mesoderm in the area pellucida (bright area) and the area opaca (dark area). These cells were of long oval shape. Short protuberances sprouted from the long axis, and the cell appeared to be a bipolar cell.

The cytoplasm was full of small and large BGs. The nucleus was in the center of the cell and of an oval shape. It was a special kind of mesodermal cell and was named *PV blast* because it formed a PV in a later stage.

4. Ten hours: The PV blasts were arranged in a line, and formed a string-like body by connecting themselves with the cytoprocesses from the long axes of the cells. There were cell nuclei in the center of the cells, and there were BGs in the string-like body. This string-like body of the PV blasts was named Pre-PV because it differentiated and developed to become a PV.
5. Fifteen to sixteen hours: The pre-PV differentiated so fast that striking transformation in shape occurred. The partitions connecting the PV blasts disappeared, and the cellular membranes formed the wall of the PV. Oval-shaped nuclei were in the center of the PV. This was named *proto-PV*.

The fact that the pre-PV was developed in the period of 10—15 hours of incubation before any other organs or cells except the germ layers were developed suggested the major role of the PVS in cell differentiation, prototype formation, and systematizations of various organs.

6. Twenty hours: The proto-PV continued to develop and extended to the area opaca via the area pellucida.

Mesenchymal cells differentiated from the mesoderm gathered in the area opaca, surrounded the proto-PV, and formed blood islands. The liquid in the proto-PV was thought to facilitate the formation of blood islands and blood vessels.

The oval-shaped nuclei in the proto-PV elongated gradually to become *rod-shaped nuclei*, and moved from the center to the wall of the proto-PV, and the cells became endothelial cells. In this way, *primo subvessels* were formed.

7. Twenty to twenty-seven hours: The wall of the blood vessel began to be formed from the blood islands symmetrically around the primo subvessel located in the middle. The blood vessel surrounded the primo subvessel, which would become an interior PV later. (The primo subvessel was formed first, and then the blood

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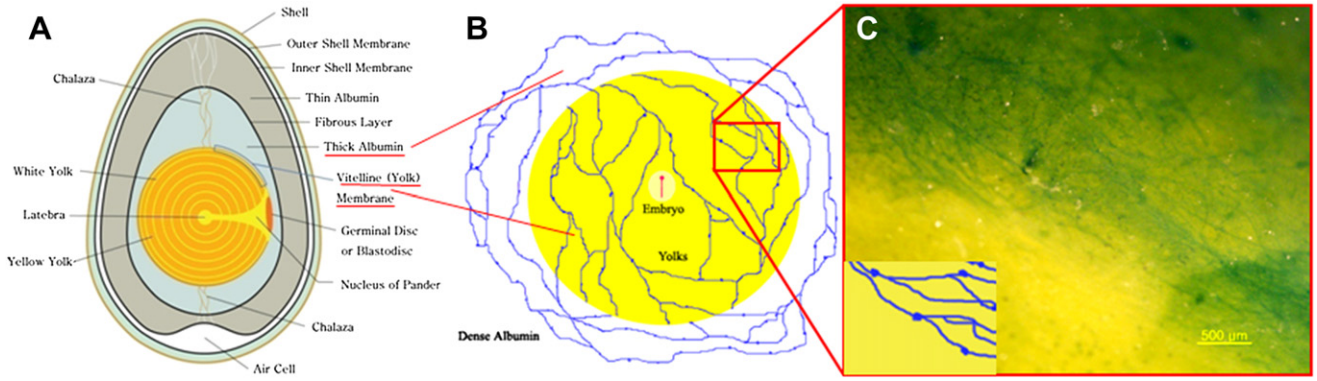

Figure 1 (A) Diagram of a hen's egg in longitudinal section. (After Lillie.) (B) Trypan blue staining on the vitelline revealed a complicated network on the vitelline and white thick albumin and some dots, which seemed to be DNA-containing bodies. (C) Magnified view of the squared region in (B).These curves and dots might be related to the PVS. The egg was incubated for 16—24 hours. In stages 4—7 according to Hamburger and Hamilton's criteria. (SY Lee, Master's thesis, Seoul National University, 2010.)

vessel started to surround it, and finally the complex of the blood vessel and the interior PV floating in the blood flow was completed. —Translator's note.)

8. From 27 to 48 hours: New primo subvessels began to be formed. Their differentiations and developments were similar to those already formed. The earlier formed subvessel and those formed later made up a bundle of multi-subvessels which was a PV.
9. From 45 hours to 5 days: PVs in various stages of formation were observable.

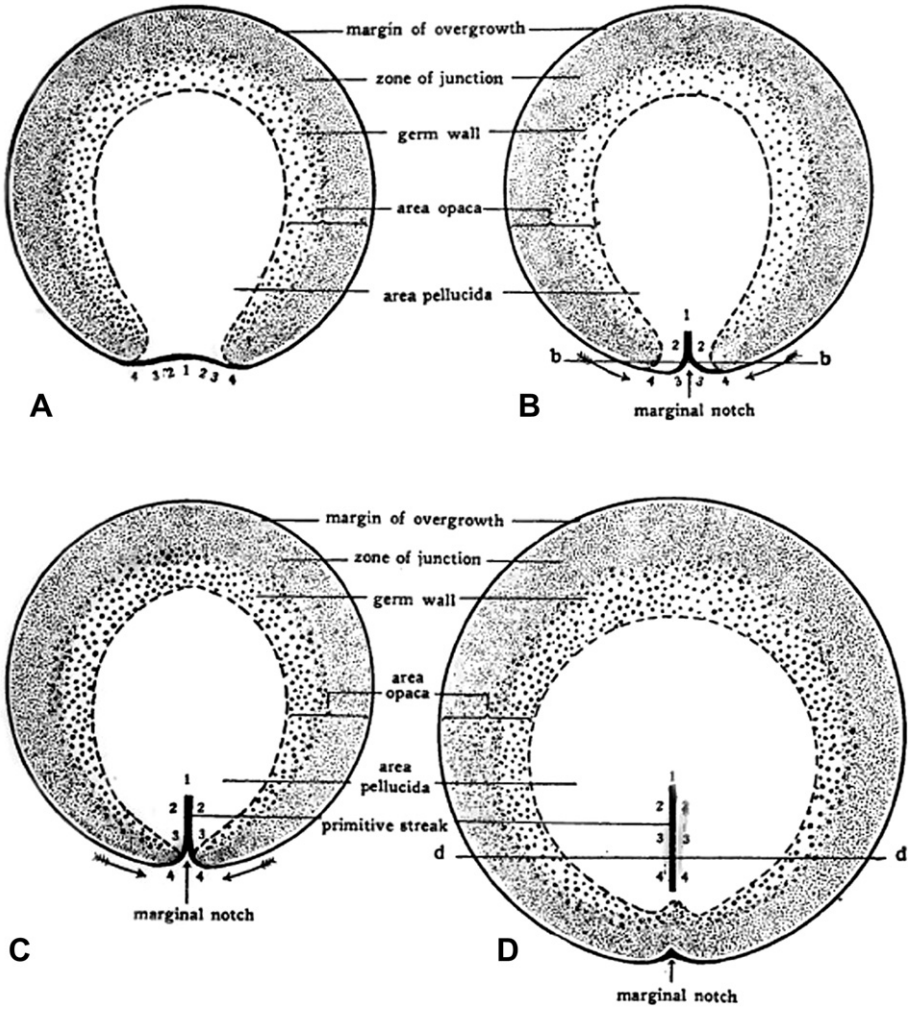

Figure 2 Schematic diagrams to illustrate the concrescence theory of the origin of the primitive streak.

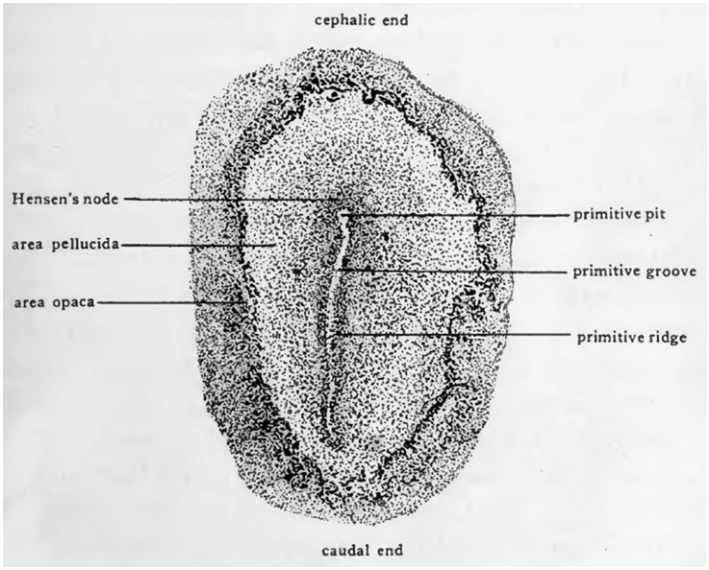

Figure 3 Dorsal view (× 14) of entire chick embryo in the primitive streak stage (about 16 hours of incubation).

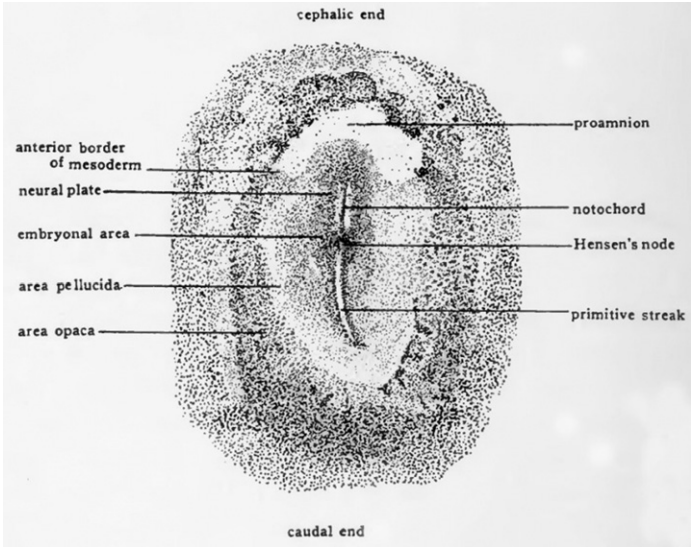

Figure 4 Dorsal view (× 14) of entire chick embryo of 18 hours incubation.

Summary: The PV was developed earlier than blood vessels or nerves, and the speed of differentiation and shape formation were faster than those of blood or nerves. (The term "primo" was adopted in 2010 to signify the earlier formation of the PVS than the blood vessels or the nerves. Another reason for the terminology "Primo" was Kim's claim that the PVS was the most essential system for regeneration of organs and general health. —Translator's note.)

The developmental stages: PV blast (7—8 hours), Pre-PV (10 hours), Proto-PV (15 hours), Primo subvessel (20—28 hours), PV (27—48 hours), complete formation of the PV (48^{th} hour). The developmental processes of the PVS were different from those of blood or lymph vessels.

Note: It was noticeable that the BGs in the PV were also observed in the eggs even before incubation. (BGs seem to be DNA0containing granules. —Translator's note.)

Here, some figures have been added by the translator for the convenience of readers.

Figures 2—13 were copied from Bradley M. Patten, *The Early Embryology of the Chick*. Philadelphia: P. Blakiston's Son and Co., 1920.

2. Comparative biological study

The developmental study hinted to us the earlier appearance of the PVS in evolution, which prompted us to investigate the existence of the PVS in animals other than mammals.

1. Vertebrate (fowls (avian), reptiles, amphibia, fish): The presence of the PVS was confirmed. The structure of

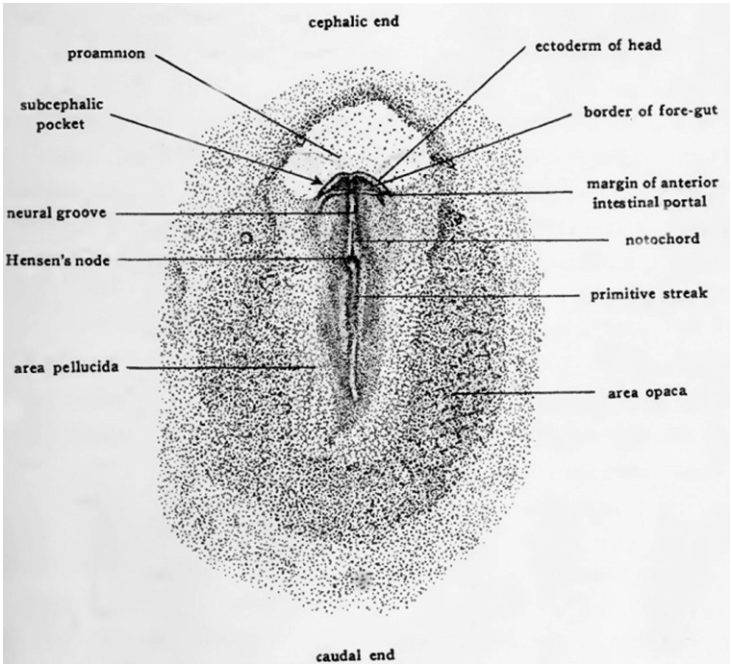

Figure 5 Dorsal view (× 14) of entire chick embryo of about 21 hours incubation.

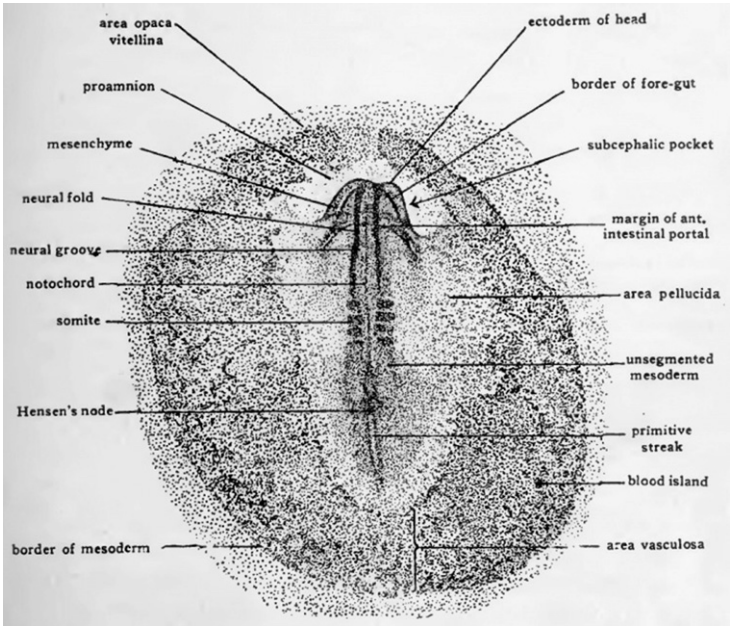

Figure 6 Dorsal view (× 14) of entire chick embryo having 4 pairs of mesodermic somites (about 24 hours incubation).

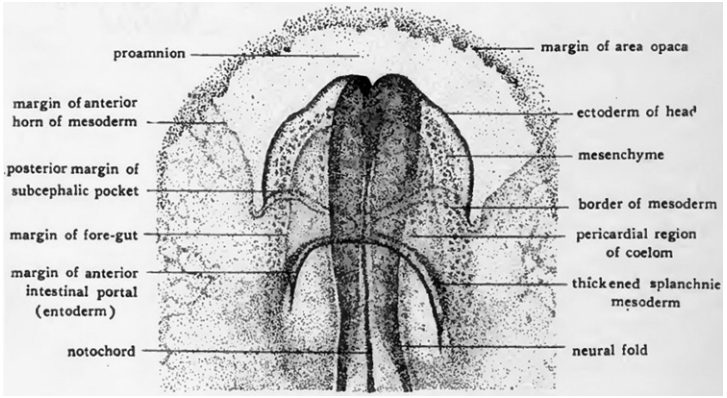

Figure 7 Ventral view (× 37) of cephalic region of chick embryo having 5 pairs of somites (about 25-26 hours of incubation).

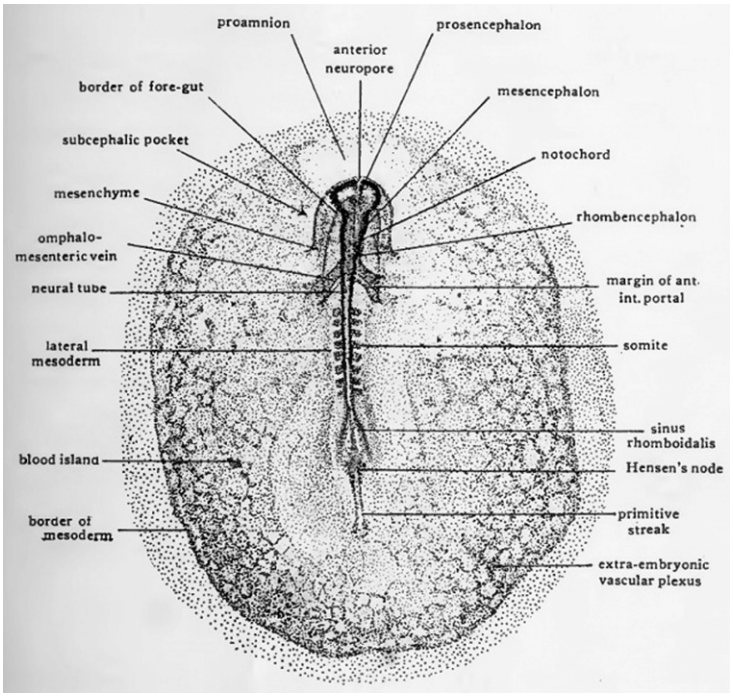

Figure 8 Dorsal view (× 14) of entire chick embryo having 8 pairs of somites (about 27-28 hours incubation).

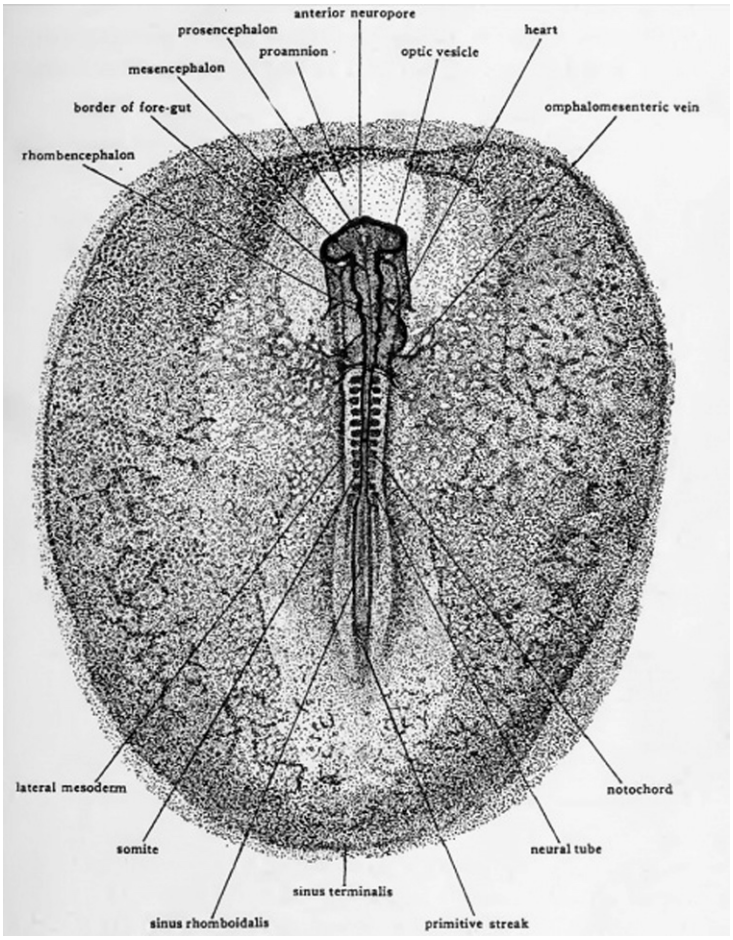

Figure 9 Dorsal view (× 14) of an entire chick embryo of 12 somites (about 33 hours incubation).

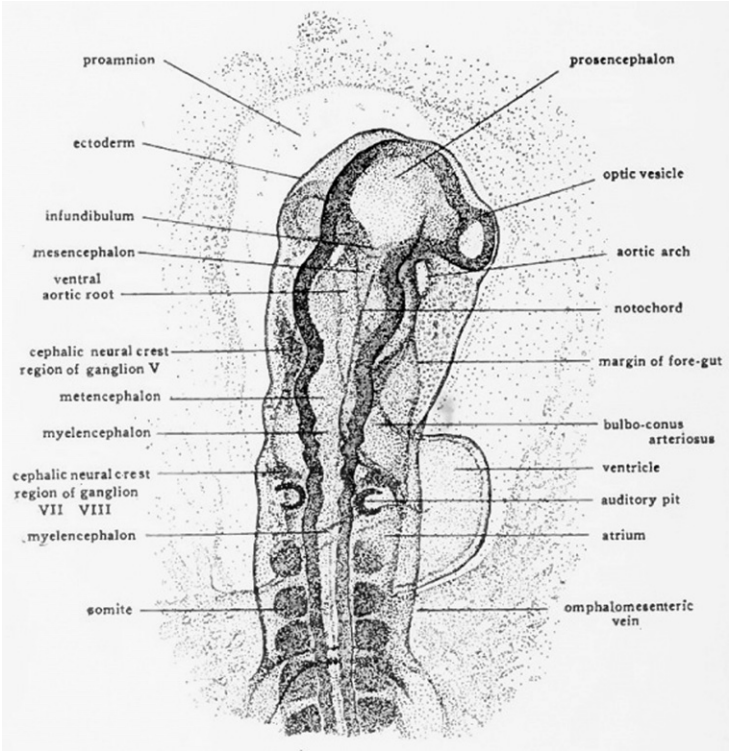

Figure 10 Dorsal view (× 45) of head and heart region of a chick embryo of 17 somites (38-39 hours incubation).

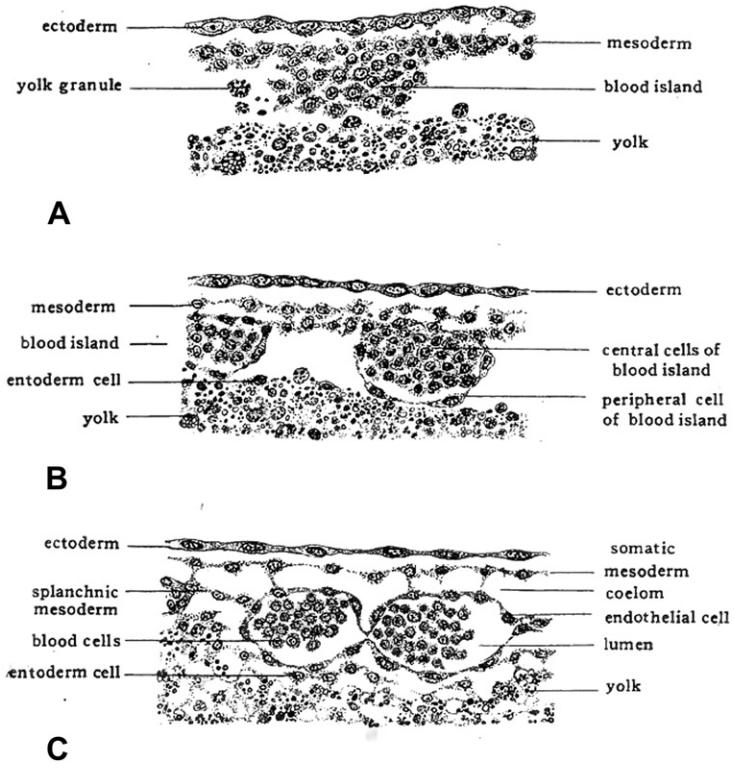

Figure 11 Drawings to show the cellular organization of blood islands at three stages in their differentiation.

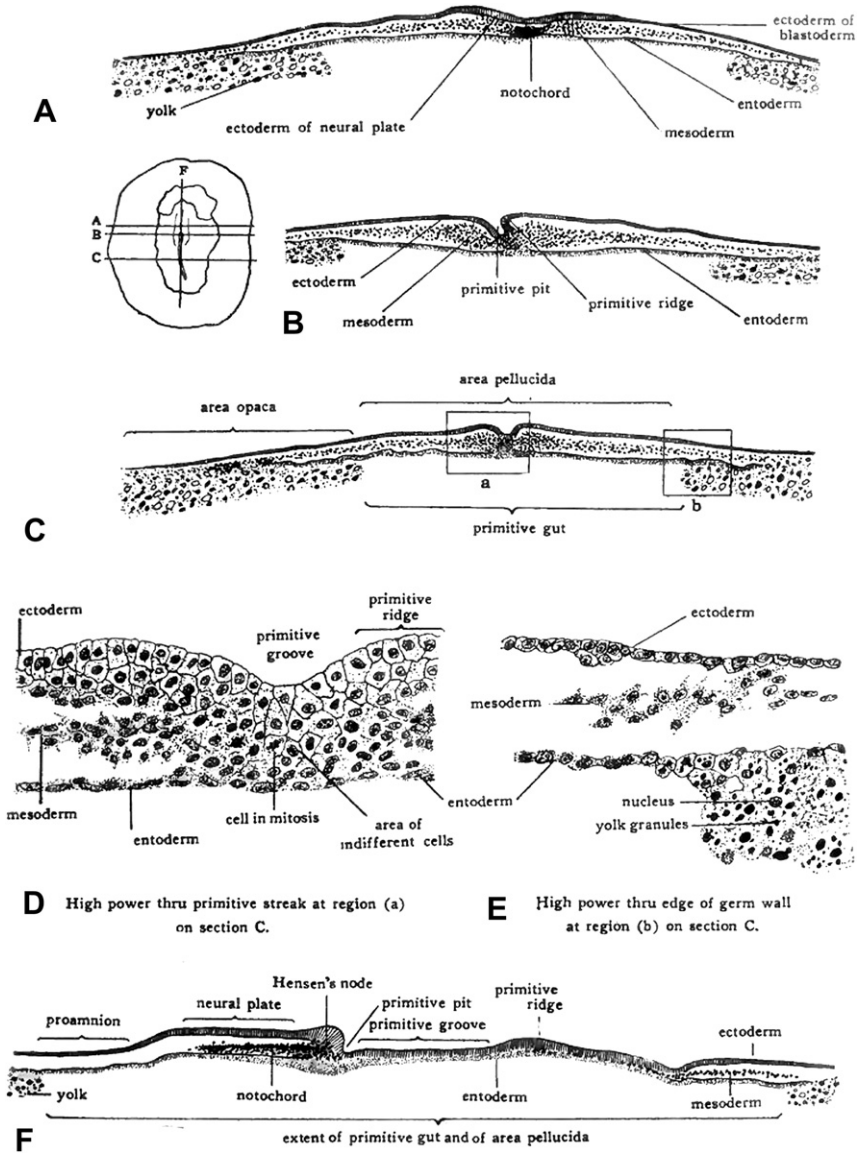

Figure 12 Sections of 18-hour chick. The location of each section is indicated by a line drawn on a small outline sketch of an entire embryo of corresponding age. The letters affixied to the lines indicating the location of the sections correspond with the letters designating the section diagrams. Each germ layer is represented by a different conventional scheme: ectoderm by vertical hatching; entoderm by fine stippling backed by a single line; and the cells of the mesoderm which at this stage do not form a coherent layer, by heavy angular dots. (A) diagram of transverse section through notochord. (B) diagram of transverse section through primitive pit. (C) diagram of transverse section through primitive streak. (D) drawing showing cellular structure in primitive streak region. (E) drawing showing cellular structure at inner margin of germ wall. (F) diagram of median longitudinal section passing through notochord and primitive streak.

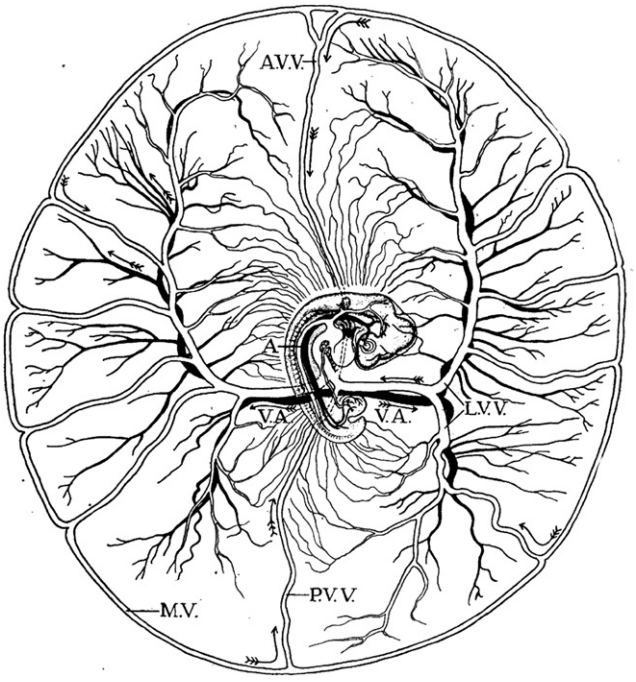

Figure 13 Diagram to show course of vitelline circulation in chick of about four days (96 hours). The direction of blood flow is indicated by arrows. Abbreviations: A, dorsal aorta; A.V.V., anterior vitelline vein; L.V.V., lateral vitelline vein; M.V., marginal vein (sinus terminalis); P.V.V., posterior vitelline vein; V.A., vitelline artery.

the PV was more or less the same, but the nuclei of endothelial cells were bigger and more distinct than those in mammalian species. (The lengths of the rod-shaped nuclei of the endothelial nuclei were 12—20 μm. —Translator's note.) The structure of the primo node was much simpler, and there were congregations of bright cells and BGs in the node.

2. Invertebrate (coelenterate): A hydra has a PV running in the coelenteron, and the branches of the PV go into the ectoderm and endoderm.
3. All multicell animals appeared to have a PVS.
4. Plants also appear to have a PVS. [For example, a sunflower (*Helianthus annuus* L.) root was mentioned by Kim in another report. —Translator's note.]