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Formation of coronary arteries sprouting from the primitive aortic sinus wall of the chick embryo

E. Aikawa and J. Kawano[1]

Department of Anatomy, Miyazaki Medical College, Miyazaki (Japan 889-16), 30 September 1981

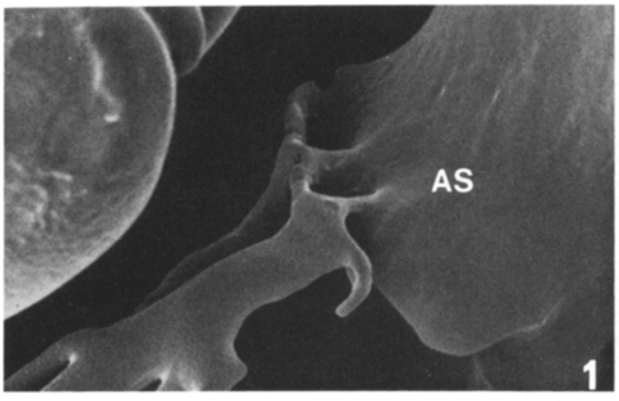
Summary. The formation of coronary arteries in chick embryos was observed by scanning electron microscopy on injected casts as well as by transmission election microscopy. Usually, 2-4 primitive coronary arteries appear from the right aortic sinus below the level of the cusp margin, and 1-3 from the left one. As development proceeds, the arteries are generally reduced in number to form a single definitive coronary artery on each side. Canalization of the arteries seems to take place by partially degenerative changes of the primordia.

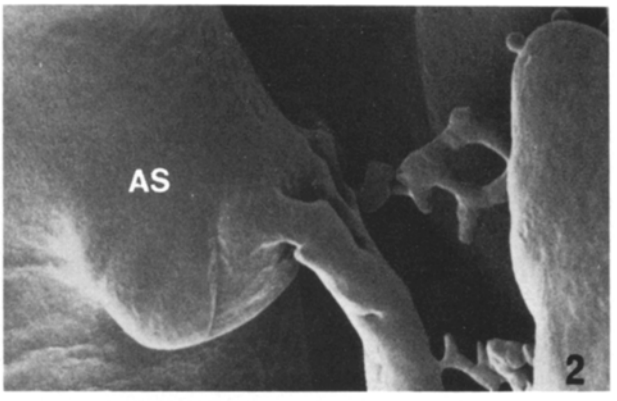
The mechanism of the formation of coronary arteries is considered to be common to many different species of animals, especially mammalian and avian embryos[2]. Evidence is available showing that the coronary arteries first appear as a solid sprout of endothelium from the aortic sinus wall, after the veins are well established. Shortly thereafter, following canalization of the solid sprout, communication with the pre-existing capillaries is established to form the primitive coronary arterial system[3-9]. The present investigation was undertaken to determine whether the number of the coronary arteries initially is one on each side only, and to clarify the sprouting mechanism leading to the formation of coronary arteries from the aortic sinus.

Material and methods. White Leghorn chick embryos ranging from stage 29 to stage 40[10] were used. By the use of vascular casts combined with scanning electron microscopy (SEM), it was possible to obtain detailed information on the microvasculature. To make vascular casts, chick embryos were initially perfused from the heart with physiological saline, followed by injection with Mercox[11]. After the injected resin had hardened, the specimens were placed in a strong solution of KOH to remove the soft tissue. Vascular casts were cleaned, dried, coated with Pt-Pd, and viewed on a JSM-F15 SEM. For transmission electron microscopy (TEM), embryos were perfused with a fixative consisting of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer. Proximal parts of aorta were removed into the same fixative for 2 h at 4 °C. They were post-fixed with 1% OsO_4 for 2 h, dehydrated with ethanol, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead hydroxide, and examined on a JEM-200CX electron microscope.

Results and discussion. Observation of the vascular casts obtained from the embryos up to stage 30 revealed no vascular branches from the aortic sinus on either side, though a part of the capillary network in the cardiac wall is located in the vicinity of the aortic sinuses. At stage 31, primitive coronary areries were found to first arise slightly below the level of the free margin of the excavating aortic cusp. The general view of the cast observed with SEM is shown in figures 1 and 2. 3 primitive coronary arteries sprout from the right aortic sinus and connect with each other (fig. 1). The same pattern is seen on the left (fig. 2). This is confirmed with the light microscopic observation of a cross section of the aortic sinus wall (fig. 4). Figure 3 (stage 35) shows that a single coronary artery arises from the aortic sinus on both the right and left sides. At stages 31-32 (7-7.5 days) the primitive coronary arteries on the right side are from 2 to 4 in number and vary in size, and from 1 to 3 in number on the left side. Concerning this problem, recently Rychter and Ostadal[8], using specimens injected with Indian ink, stated that communication with the aortic cavity doubled symmetrically in the primordia of the coronary arteries in 3 out of 6 cases at 8 days of incubation. However, as far as our investigation is

Figure 1. SEM view of a vascular cast of the right coronary arteries from the chick embryo at stage 31. 3 primitive coronary arteries arise from the aortic sinus (AS) to communicate each other. They are irregular in shape and size. × 300.
Figure 2. SEM view of a cast of the left coronary arteries at stage 31. 3 primitive coronary arteries from the aortic sinus (AS) communicate to from a single descending artery. × 300.
Figure 3. SEM view of a vascular cast of coronary arteries from the chick embryo at stage 35. From both the right and left aortic sinuses (AS), a single coronary artery appears respectively. Shallow pits (arrows) are detected on the cast of the original part of the coronary arteries. RCA, right coronary artery; LCA, left coronary artery. × 60.
Figure 4. A 1 μm cross secrtion of the right aortic sinus at stage 31. 3 primitive coronary arteries (arrows) are detected in the aortic wall. Capillaries (C) which developed in the cardiac wall are located in the vicinity of the arteries. Toluidin blue stain. AL, aortic lumen. × 200.
Figure 5. TEM view of cross section of the aortic sinus from the embryo at stage 31. One of the right primitive coronary arteries is demonstrated. The small lumen is surrounded by irregularly formed primitive endothelial cells with varied electron opacity. AL, aortic lumen. × 1100.
Figure 6. TEM view obtained at stage 30. The solid cell mass arranged vertically to the aortic luminar surface is one of the primordia of the primitive coronary arteries. Large pale cells (PC) are surrrounded by irregularly shaped dark cells with some vacuoles of various sizes. Some of these cells are thought to be degenerative cells because of the obscurity of their organelles and their electron opacity. AL, aortic lumen; C, capillary. × 2400.


AS
1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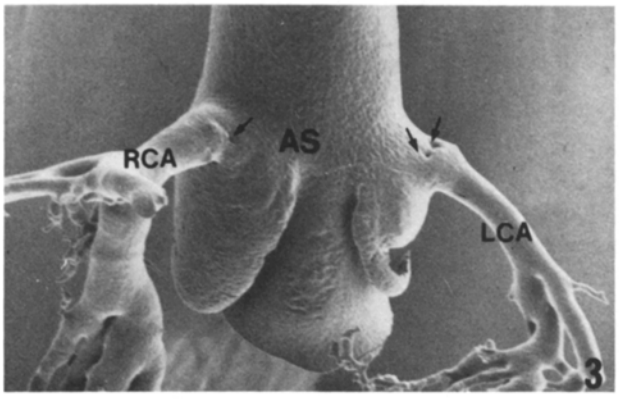
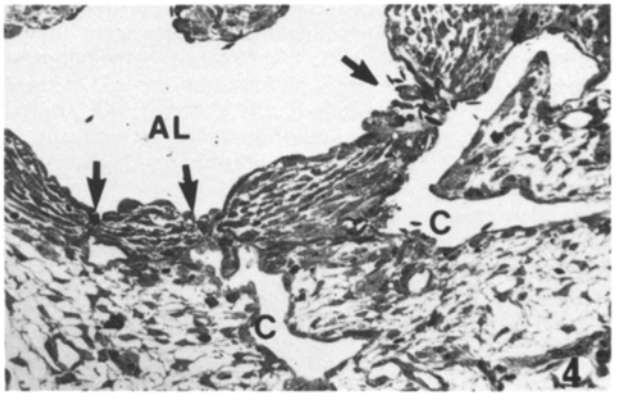
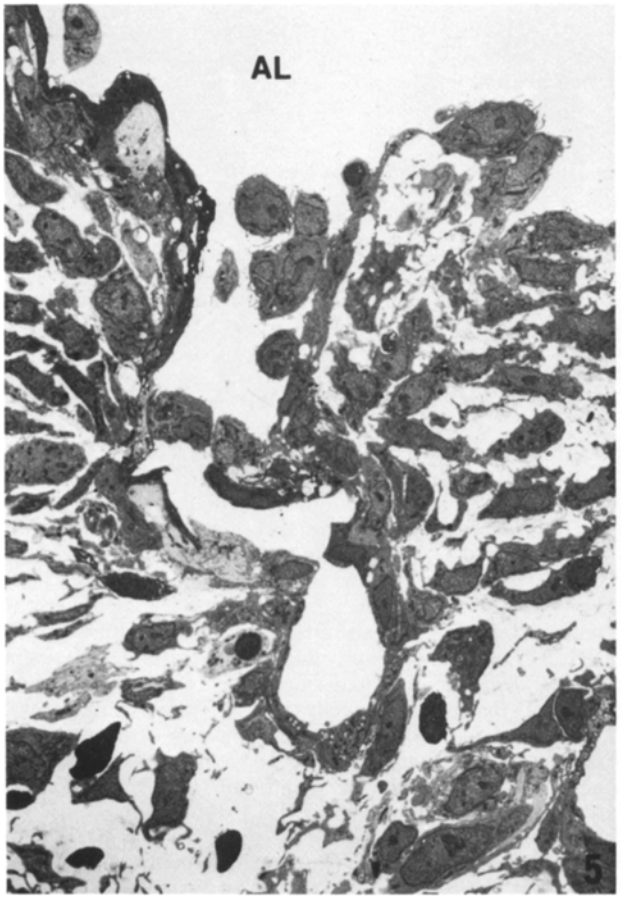
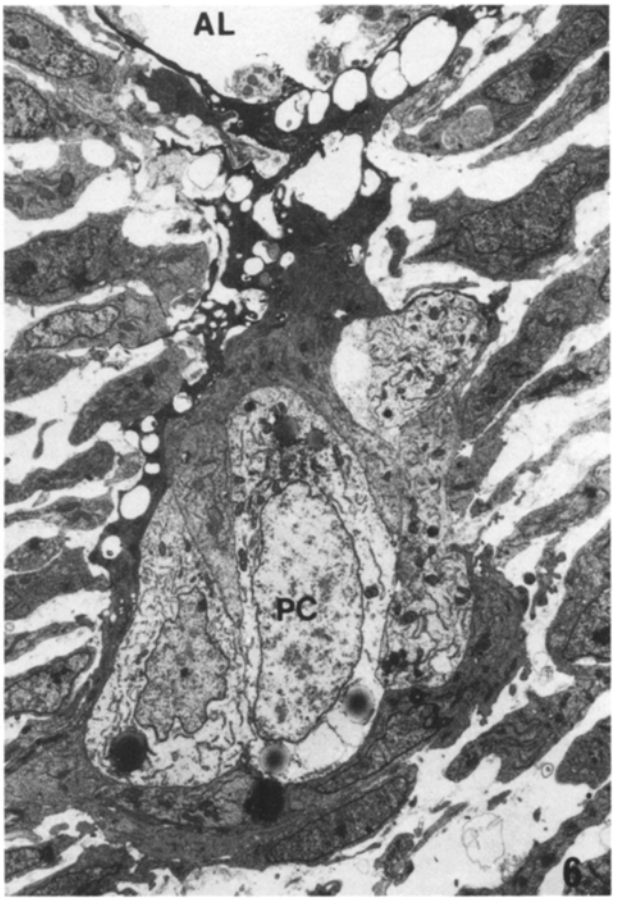

RCA
AS
LCA
3


AL
C
C
4


AL
5


AL
PC
6

concerned, at least 2 primitive coronary arteries are detected, especially on the right side in all the cases observed. These primitive coronary arteries decrease in number in the later stages, when they seem to fuse side-to-side to form a single coronary artery, because the remaining shallow pits are observed on the casts at the next stage (fig. 3). However, the fusing process has not been clarified, and is now under investigation. These phenomena in the development of coronary arteries should be of much help in understanding the variations of adult coronary arteries[12]. Contrary to some researchers' descriptions[3,9], our research showed that the sprouting of the left primitive coronary arteries is not earlier than the right. Figure 5 shows a general TEM view indicating one of the primitive right coronary arteries observed at stage 31. The aortic sinus wall is a multilayered structure (7-10 layers) of alternating primitive vascular cells and intercellular layers. The surface of polymorphic endothelial cells displays occasional microvilli. Further, a narrow vascular lumen communicating with the aortic lumen is observed in the sinus wall. The structure corresponds to the primitive coronary artery observed on the cast at stage 31. The primitive endothelial cells covering the arterial lumen take irregular forms and are arranged vertically to the sinus wall. Some of these are presumably degenerating cells as suggested by their electron opacity. Figure 6, obtained from the embryo at late stage 30, is very suggestive with respect to the process of formation of the primitive coronary arteries. The large pale cells, surrounded by irregularly shaped electron opaque cells containing various sizes of vacuoles, are localized vertically to the aortic luminar surface, and as a result intersect the circularly arranged cell layers. It cannot be decided definitely whether the pale cells and dark ones are derived from the aortic endothelium, since their cellular characteristics differ from the endothelial cells observed on the aortic surface. It seems likely that the canalization of the primordium follows cell death. As a result, canalized primordia fuse with the pre-existing capillaries in an end-to-end or end-to-side fashion to form the primitive coronary arteries. It remains to be shown whether or not the pre-existing capillaries inductively participate in the formation of the primordium of the primitive coronary arteries.

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On the problem of linear incorporation of amino acids into cell protein

D.N. Wheatley[1]

Cell Pathology Laboratory, Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen AB92ZD (Scotland), 6 July 1981

Summary. Incorporation of amino acids into mammalian cell protein shows immediate linear kinetics when measured at intervals as small as 5 sec. Free amino acids equilibrate instantaneously across the cell membrane, acting as the precursor supply. Glycine, which does not initially show linear kinetics, may have a significant endogenous precursor supply, but this is not the acid-soluble pool.

A simple but unexplained observation is that amino acids are incorporated into protein in cultured mammalian cells with linear kinetics[2-4]. Culture conditions facilitate studies on initial rate of incorporation because cells are bathed in a plentiful supply of medium, unlike the in vivo situation where extracellular fluids equilibrate less rapidly. No part of a monolayer cell is more than about 1 µm from the medium (or about 7 µm for a suspension cultured cell), allowing instantaneous equilibration of free amino acid molecules across the cell membrane[7-9]. It is noteworthy that other free-living organisms such as *E. coli*[5] and *Tetrahymena*[6] show linear incorporation kinetics into protein.

Where delays in incorporation occur, these are often related to the size of the acid-soluble pool and the speed with which incoming amino acids equilibrate with it. Substantial evidence[10,11] now shows that this pool does not play a precursor role in protein synthesis. Apparently contradictory evidence of Robinson[12], after correction of technical short-comings, actually corroborates that the pool is not the precursor supply[11]. The controversy is kept alive by the suggestion[13,14] that only a small part of the pool, i.e. a rapidly equilibrating 'membrane pool', provides precursors. Since the intracellular acid-soluble pool of phenylalanine or leucine contains sufficient molecules to sustain protein synthesis for only 5 min[8], the question is raised of how strict is the initial linearity of incorporation of amino acids into protein? This paper demonstrates that a) linear kinetics occur immediately after the addition of a labeled amino acid, and b) the acid-soluble pool is not directly involved, even in the case of glycine where delay occurs before linear kinetics are established[7]. Experiments reported here involved HeLa S-3, 3T3 and BHK21/C13 cell cultures.

Results. 1. *Preloading of cells.* The acid-soluble pool of an amino acid expands with increasing external concentration[14,15]. By preloading cells, any precursor component of the pool would be more evident because a) incorporation rates rise with availability of exogenous amino acids[10,14] and b) an intracellular or 'membrane' pool would increase to maintain its equilibrium with the medium. Cells were preloaded with 0.1, 1.0 and 10×10^{-3} M leucine in basal Eagle's medium + 10% neonatal calf serum for 30 min, spun out and resuspended in medium with 10^{-4} M leucine