STUDIES ON THE BLOOD VESSELS IN THE MEMBRANES OF CHICK EMBRYOS

PART III. ANATOMY AND PHYSIOLOGY OF THE BLOOD VESSELS AT DIFFERENT AGES*

BY A. E. COHN, M.D., AND FRITZ LANGE,† M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

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The development of the vascular system of the membranes of chick embryos passes through a definite cycle. It can be followed from its inception in the blood islands, through the stages in which the blood vessels are finally developed, to the time when it is discarded at the time of hatching. It ought to be possible therefore to correlate changes in function with the anatomical changes. We hoped to obtain an insight into the alterations which take place in both, and to do this the more readily owing to the short duration of the whole cycle.

Physiological Experiments

The irritability of the vessels was studied by applying stimuli of the same strength to them from their inception to the time of hatching. The experiments were carried out in eggs that had been opened as in Parts I and II of these studies.

It was necessary to introduce a slight modification in technique in the case of older embryos for in them it is more difficult to remove the membrane than in embryos 5 days old. The eggs were therefore kept on end, the air-chamber upwards, for 12 hours before the beginning of the experiment. With practise it became easy after opening the shell in the region of the air-chamber, to remove the shell membrane with fine forceps and scissors. The blood vessels then are as easy to approach as they are in younger embryos in which they are disposed along

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† Privatdocent at the First Medical Clinic, University of Munich. Associate in Medicine, The Rockefeller Institute for Medical Research, 1928–29.

the long axis of the egg. The stimuli employed were a faradic current of known strength, sodium iodide, ammonia and mechanical stimulation with the use of a glass rod. As in our earlier experiments we ascertained which strength of stimulus it was necessary to employ in order to secure stasis in an egg after 3 days of incubation. We then chose a stimulus based on this, but weaker, maintaining all the stimuli which we employed at the same strength for all other ages. On account of the similarity of our observations it is necessary to describe only our results.

At the time when there are only blood islands and no regular flow of blood, contraction and dilatation do not occur. At this early age the occurrence of coagulation as the result of the use of chemicals in strong concentrations can naturally not be observed. Our observations begin therefore with the behavior of blood vessels at the time when a regular rate of flow begins, namely, on the 3rd day. All stimuli which bring about stasis in arteries of the membrane on the 3rd day of incubation produce the same dilatation in the same length of time in arteries of the same size and in the same way in all eggs of all later ages. The criterion of a strong stimulus has been described in previous sections of these papers and may be defined as follows: In the case of a faradic current it is one which on moist lips causes an unpleasant sensation of burning. In the case of sodium iodide a concentration of 6 per cent in the amount of 0.1 cc. and in the case of ammonia a 5 per cent solution of the same amount. We chose a weaker current, as has been said, one which in small arteries causes contraction and studied the reaction to it through all ages up to the 19th day, that is to say, shortly before hatching of the egg. In all stages of development the effect of stimulation of arteries of the same caliber is the same, namely, contraction.

A difference in the manner of reaction is always visible. Capillaries react to weaker stimuli than do arteries. In this sense specimens which are younger differ from older ones. In specimens 3 and 4 days old, all the vessels respond to stimuli which in older embryos affect only those vessels which have the same small dimensions as the young ones. The larger vessels in older embryos require stronger or longer stimuli. For example, stasis was observed in all arteries in 3 to 4 day old embryos as the result of applying an electrical stimulus of a certain strength for 20 seconds. In specimens 18 days old on the contrary this stimulus applied to large arteries required twice the dura-

tion, namely, 40 seconds or a correspondingly stronger stimulus for 20 seconds to bring about the same effect. This striking difference is to be explained in terms of histology.

Anatomical Investigations

There are satisfactory investigations by Popoff (1) and H. Virchow (2) on the distribution and branching of blood vessels of the vascular membrane of chick embryos at various ages, but a comparative histological study of these vessels at all stages of development has not so far been published. In especial nothing is known on the presence of elastic tissue nor of the appearance of degeneration in the older aged groups. In the very early stages of development studies have been made by Sabin (3) both in living preparations and histologically. According to her investigations a continuous differentiation of mesenchymal cells to angioblasts takes place in the first 2 days. These cells develop first in the membranes which are of interest to us. Angioblasts differentiate further during the course of the 3rd and 4th days of incubation. The angioblasts give rise to endothelium, blood plasma and blood islands. Only those structures are called blood islands which develop hemoglobin and become erythroblasts. The lumina of the blood vessels come into being through a process of cytolysis either in the centers of the cell masses or in the cytoplasm of single cells.

Our histological description begins at the 3rd day of incubation when the flow of blood in the vascular membrane has become established.

Technique.—Freshly opened eggs were fixed in formalin, Mueller formol or Zenker's solution. Blocks of paraffin or of gelatin were then made which were cut in series from the marginal veins to the embryo. Specimens of various ages were so prepared, cut either horizontally or transversely. They were stained with hematoxylin and Van Gieson's solution, resorcin-fuchsin and Sudan hemalum.

Description of Embryos 4 Days Old.—Radiating from the embryo there are ridges and folds which press into the yolk. In these folds the blood vessels run. The arteries lie in the folds nearer the yolk. They consist of single rows of flat or spindle cells, the circumference of the wall being the length of 10 to 12 cells. In the surrounding area there is a loose mesh network in which there are large nuclei. These cells do not belong to the arteries since they are also to be found without relation to them. Within the vessels there are free nucleated cells either round or spindle-shaped depending upon the aspect which they present. Laterally from

the arteries lie two veins. In comparison with the arteries they have a characteristic form. They are polygonal or flattened and adapt their shape to the space which is available, in this case, half surrounding an artery. Their wall is thin and consists of a single layer of cells. Arteries and veins are immediately juxtaposed so that at such places two layers of flat cells are to be found. There are no elastic fibers and no collagen. All vessels that belong to the yolk have the same form and are to be distinguished only by their size.

Description of Embryos 10 Days Old. Vessels at a Distance from the Embryo.—In the interval the dimensions of the arteries have increased to a five-fold extent. The wall turned towards the yolk consists of a single layer of cells. They contain a light staining protoplasm. The nucleus stains darker and contains many dark points arranged about a larger, more compact, area within. The relation of arteries and veins is less intimate; the veins run, at least in part, an isolated course.

Vessels near the Embryo.—Arteries have come to differ from veins through the distinct difference in thickness of their walls. On cross section the arteries appear to be round in contrast to the veins which are capable of taking on a variety of forms. The veins surround the arteries to the extent of a half to three-quarters of their circumference. The artery is embedded in the loose web-like tissue of the embryonic membranes. Their walls are about 5 cells thick. Their arrangement is lamellar. The nuclei are arranged in the long axis. The protoplasm appears more concentrated and is less in amount. The inner layer consists of somewhat taller endothelium. Between the cells delicate fibers stained bright red with Van Gieson's solution are to be found. There are no elastic fibers and no fat. The veins exhibit, surrounding the single row of cells of which they are formed, a layer of cells somewhat loosely meshed, the cells being large and composed of light staining protoplasm. Between the largest and the smallest arteries transitions occur ranging in dimension from arteries presenting two to three layers of cells to those of the largest size. The arrangement is always circular and never longitudinal. The smallest blood vessels, namely capillaries, often appear close to arteries, touching their outermost layers, but do not penetrate their walls.

Description of Embryos 14 Days Old. Vessels at a Distance from the Embryo.—The smallest arteries are built like capillaries and are to be distinguished from them only according to their size. They are of exactly the same form and structure as were those described in embryos of an earlier age. Nearer the embryo there are likewise to be found larger arteries of 2 to 5 layers.

Vessels near the Embryo.—A large artery of about 12 layers is to be found, the lumen being lined with a single layer of endothelium. Surrounding this is a longitudinal layer three cells thick. Beyond is a circular layer seven cells thick. Outside the vessel there lie connective tissue cells irregularly distributed and loose meshed, the cells containing small nuclei. The protoplasm of the cells in the arterial wall stains brown with hematoxylin and Van Gieson. These are young muscle cells. Distributed among them there are a few collagen fibers stained pale red. In the loose meshed tissue which is to be regarded as the adventitia there are

a few collagen fibers but there are no elastic fibers and there is no fat. The veins have a larger lumen than the arteries and are built of single layers of endothelium and about two layers of cells. The cell layers appear to be looser meshed than in arteries.

Embryos 18 Days Old. Description of the Vessels Far Removed from the Embryo.—Arteries built like capillaries are to be found as well as small arteries such as have already been described.

Vessels near the Embryo.—The arterial trunk described in embryos 14 days old is to be found also here. The wall has become thicker. The nuclei are well stained and their structure unchanged. There is no evidence of nuclear degeneration, decrease in the size of the nuclei nor indeed of any other change. There is no fat in the wall nor any elastic fibers.

At the Site of Transition to the Embryo.—The form of the artery is unchanged. There appear to be more collagen fibers. Sections stained with resorcin-fuchsin exhibit stoutly developed elastic fibers. In the outer third of the muscle layer there lies a broad band of elastic fibers passing in part into the adventitia. This zone consists of about five layers of short twisted elastic fibers which form a meshwork. There is a second layer of elastic fibers very fine in structure, which forms the internal elastic layer close under the single layer of endothelial cells. This structure also is not closely organized, but consists of single short slightly curved fibers which run circularly. Neither fat nor any other form of degenerative substance is to be found. This part of the artery exhibiting elastic fibers should no doubt be reckoned as being part of the embryo. This is the portion in which, as has been described in Part I, nerve fibers were found.

In the embryo itself in the early stages there are definite elastic rings in arteries of very much smaller dimensions than in the vascular membrane. The proof that elastic fibers occur in arteries within the embryo, whereas in those without there are none, is certain since in one and the same section both the embryo itself and the vascular membrane are frequently found.

SUMMARY AND DISCUSSION

On the basis of the anatomical studies presented the following inferences or conclusions are drawn.

1. In the course of development there appear in the vascular membranes of chick embryos arterial vessels of all calibers, namely, capillaries, small arteries with 2 or 3 cell layers, and large arteries formed of endothelium, longitudinal and circular layers of muscle and adventitia.

2. In none of the stages are elastic fibers developed. Only in the most central portion of the umbilical artery, in that portion namely

which is to be regarded as belonging to the embryo, are elastic fibers discoverable.

3. The structure of capillaries is histologically the same at all stages. The small arteries of embryos 10 days old resemble histologically those of 18. At no stage of development are appearances of degeneration nor of fat to be found in arteries.

When the physiological results of our investigations are compared with the anatomical ones the following comments may be made. In respect to Paragraph 1 of the anatomical results we may remark that when we study the different forms of the wall of arterial vessels the most delicate vessels consisting of single cells exhibit the greatest irritability. Those which are built of 3 to 4 muscle layers are less irritable. Stouter vessels appearing for the first time at 10 days of incubation require stronger stimuli to bring about the same reaction. In respect to Paragraph 2 of the anatomical results we may make this comment. The absence of elastic fibers in all arteries of the embryonic membranes throughout the period of their development is important in defining a physiological property of the larger vessels.

The medium and larger vessels, beginning with the 4th day of incubation, contract differently from normal adult human arteries. In the contracted state they appear in cross section not as small replicas of larger circular structures, but take on a new form. During the course of contraction they become flat and appear band-like as would a garden hose when it is compressed by a weight. In examining a vessel so contracted one sees on rotating the vessel either a broad side or a narrow one. It is for this reason that such arteries appear alternately narrow as a line or broad as a band. It is not until the narrow artery is elevated with a hook that its uniform band-like nature becomes evident. The absence of elastic tissue, the presence of which in all probability is mainly responsible for the usual shape of arteries on cross section, permits one to see how the phenomenon which has been described may come about. Concerning Paragraph 3 of the anatomical conclusions we have this to say. According to the histological investigation a stage of degeneration is wanting in the blood vessels of the embryonic membrane in a sense in which one is accustomed to see such changes in other blood vessel systems during the course of life. On the day of hatching the constituent cells and fibers

of the arteries of all calibers are anatomically the same as in their early development. These vessels do not die as the result of aging: The nutrient fluid ceases to flow because of contraction of the umbilical vessels. The blood vessels die in complete possession of their physiological irritability and anatomical integrity. The unaltered irritability of blood vessels of the same caliber at all ages is consonant with their unaltered anatomical structure.

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