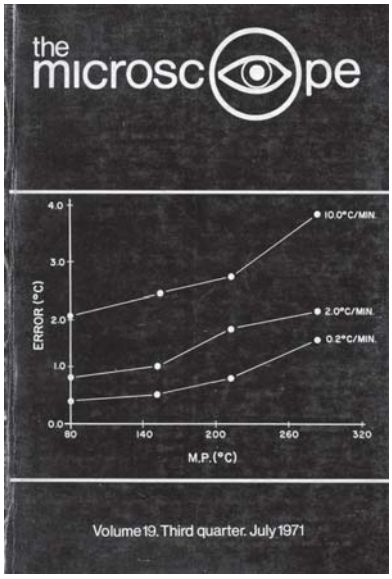
THE MICROSCOPE PAST
40 YEARS AGO

A Scanning Electron Microscope Technique for Study of the Internal Microanatomy of Embryos[1]

P.B. Armstrong
Department of Zoology, University of California*

ABSTRACT

A technique for the study of the internal anatomy of embryos with the scanning electron microscope is described. Partially sectioned paraffin embedded embryos are processed for examination with the scanning electron microscope by critical point drying, after removal of paraffin. The technique should prove useful for studying a variety of problems where knowledge of micro-anatomical relations in three dimensions is required.



INTRODUCTION

The traditional method for studying microscopic anatomy involves examining sections of the material of interest under the light microscope. A variety of techniques have been developed to aid visualization of three-dimensional organization from the study of two-dimensional sections (1), including mathematical methods of analysis and construction models (2). These methods are often tedious, rather difficult, and not always accurate. The following report describes a simple method of tissue prepartion for study with the scanning electron microscope, which results in a straightforward method for studying tissue structure in three dimensions. This method should prove a useful addition to the repertoire of techniques for studying of microanatomy and should prove especially useful in teaching microanatomy. Indeed, I have found it helpful in presenting the rather complex structure of chick embryos to students in introductory courses in embryology.

MATERIALS AND METHODS

Chick embryos were removed from the yolk using the filter-paper ring technique. After washing in Hanks solution, embryos were fixed in Karnovsky's fixative, modified according to Trelstad (3), dehydrated in a graded series of ethyl alchohol, cleared in toluene and embedded in paraffin. Embryos embedded in paraffin were sectioned on a rotary microtome to the desired depth. Sectioning was then stopped; the last sections cut were mounted on microscope slides, and the paraffin blocks containing the remainders of the embryos were saved.

These remaining portions of the embryos were then processed for examination with the scanning microscope. The paraffin blocks were melted in a paraffin oven. The embryos were gently removed from the melted paraffin with forceps and were deparaffinized in several changes of warm toluene which in turn was removed by several changes of absolute alcohol. Embryos were then transferred to amyl acetate and dried by the critical point method (4). Dried embryos were mounted for examination with the cut surface uppermost. The mounted embryos were gold-plated in a

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*Davis, CA 95616

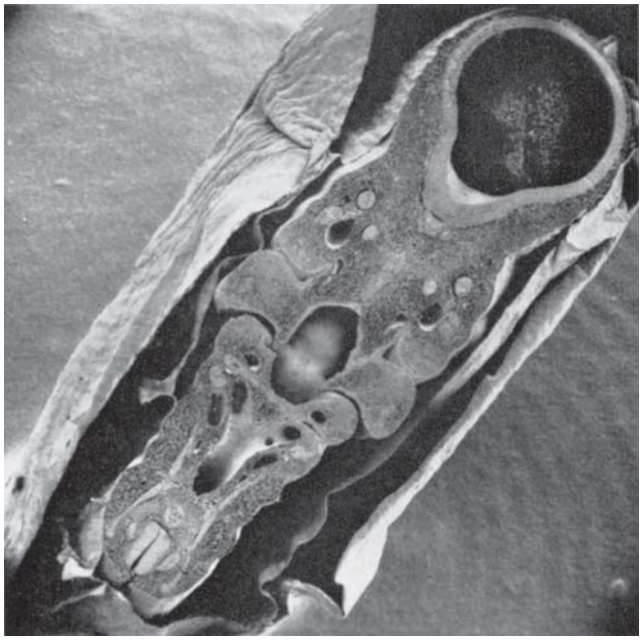

Figure 1. View of pharynx of 72-hour chick embryo. Anterior cardinal veins, aortic arches and dorsal aorta are prominent (33X magnification).

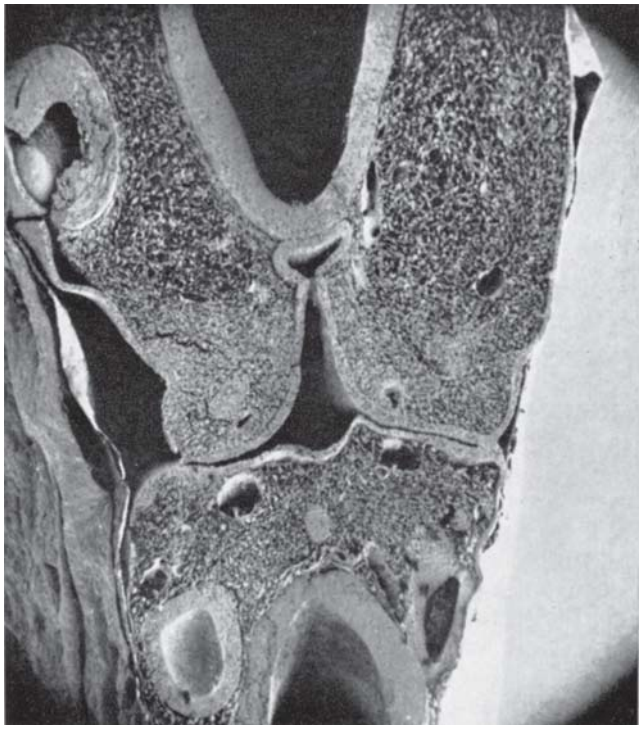

Figure 2. Forty eight-hour chick embryo at the level of the eye and otic vesicle. First and second aortic arches are visible. Rathke's pouch can be seen applied to the floor of the forebrain (112X magnification).

vacuum evaporator and were examined with a Cambridge Stereoscan scanning electron microscope. Stereomicrograph pairs were taken by tilting the specimen 9 degrees between exposures.

RESULTS

Some representative pictures of the cut surfaces of transversely sectioned chick embryos were presented in Figures 1-4. The technique discussed here is capable of resolving tissue structure and tissue type (i.e., epithelial tissue can be distinguished from mesenchyme). Cellular detail or internal cell structure is not revealed (although the scanning electron microscope is quite capable of resolving structures as small as the nucleus, the mitochondrion, or other cell organelles). The critical point drying technique utilized as the terminal step of tissue preparation prior to gold plating and examination in the microscope is employed to minimize distorton during drying. Occasionally cracking has been noted (Figure 4) but whether this occurs during drying or during mounting has not been determined.

DISCUSSION

Traditionally, anatomical structure of embryos has been studied by means of examinatioin of serial sections of embryos. Perhaps the most difficult problem faced by students of micro-anatomy is the development of an understanding of anatomical structure in three dimensions from the examination of serial two-dimensional sections. I have found scanning electron micrographs of partially sectioned embryos to be a useful addition to traditional approaches to the study and teaching of mcroscopic anatomy. The clarity, high resolution and great depth of field of scanning electron micrographs permit demonstration of features of anatomy difficult to appreciate by more traditonal methods.

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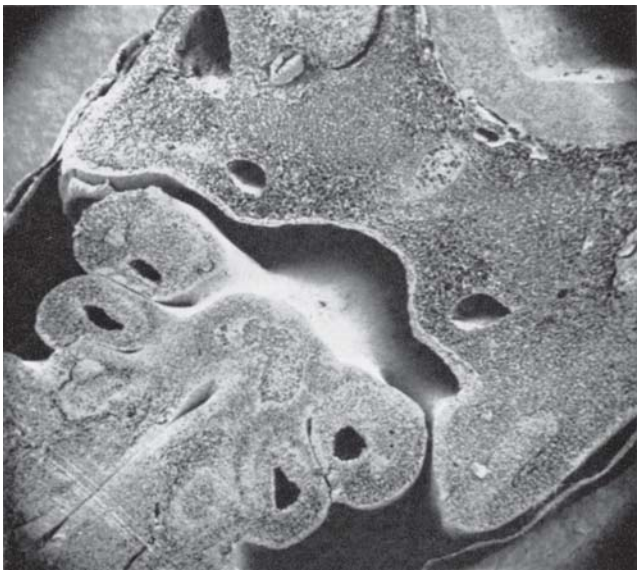

Figure 3. Section passing through floor of pharynx of 72-hour chick embryo (110X magnificaton).

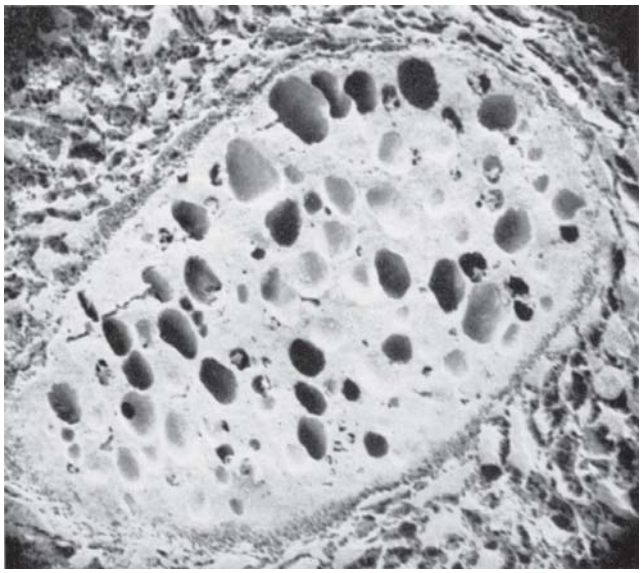

Figure 4. Enlarged view of notochord in Figure 3. At this stage in development, notochord cells contain intracellular vacuoles, visible here as empty spaces (1164X magnification).