Ultrastructural and Biochemical Observations on the Metanephros of Normal and Cultured Chick Embryos

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Summary. A comparative electron microscopical study was conducted on the metanephros from chick embryos differentiated either in shell-less culture or in ovo. Developmental characteristics were very similar in both cases. Up to stage 37 (Hamburger-Hamilton) the metanephros contained large numbers of immature nephrons; their renal corpuscles were crescent-shaped and consisted of an outer layer of flat cells and an inner one of cuboidal cells. In more advanced corpuscles also found at this stage the inner layer had formed numerous rudimentary pedicels and the tunica media of the glomerular arteriole contained juxta-glomerular cells with numerous, small, electron dense granules.

In the metanephros from embryos at stage 38 or older, large numbers of nephrons had completed their differentiation; their rounded renal corpuscles had fully differentiated podocytes with thin interdigitating pedicels and the proximal convoluted tubules had numerous apical microvilli, vesicles, vacuoles and tubular invaginations indicating an active process of resorption. These results appear to indicate that both in culture and in ovo-developed embryos, the metanephri start to function around stage 38. In the case of normal embryos this conclusion agrees with previous physiological and biochemical determinations. The injection of 20 USP parathyroid hormone into 16-day old chick embryos produced an increase in the concentration of cyclic AMP in the metanephros. This favours the idea that the regulation of kidney function by the hormone begins during the embryonic period.

Key words: Metanephros – Chicken embryo – Ultrastructure – Parathyroid hormone – Cyclic AMP.

Introduction

In birds as well as in mammals the kidney plays a role in the regulation of calcium homeostasis; it does so by varying the rate of calcium and phosphate resorption in response to parathyroid hormone stimulation (Levinsky and Davidson, 1957;
It is not known if the embryonic metanephros has similar capabilities.

The parathyroid gland appears to be active in the chick embryo (Narbaitz, 1972; Narbaitz and Gartke, 1975) and the injection of exogenous parathyroid hormone to the embryo produces a significant hypercalcemia (Narbaitz, 1975). Before suggesting the possibility that the kidney is a target organ for the hormone also during embryonic life it is necessary to find out if the chick metanephros is functional during the period in which calcium transport is more intense, i.e., after the 12th or 13th day of incubation (Johnston and Comar, 1955).

Classical studies have indicated that chick metanephros becomes functional around the 12th day of incubation (Romanoff, 1960). However, these conclusions are based only on histological observations and on experiments testing the capacity to eliminate and concentrate dyes (Romanoff, 1960). We did not find in the literature descriptions of the fine structure of the chick embryonic metanephros; we decided to attempt to fill this omission since the ultrastructural characteristics of both renal corpuscles and proximal convoluted tubules are known to be a good indication of their filtration and resorption activities respectively (Bloom and Fawcett, 1975).

The shell-less culture of chick embryos has been used successfully to clarify various aspects of calcium metabolism in the embryo (Narbaitz and Jande, 1978). Preliminary experiments at our laboratory (unpublished) have shown that the parathyroid hormone is capable of inducing in cultured embryos an increase in calcium concentration in the serum. Again in this case, it would be of interest to establish if the metanephros are adequately differentiated in the cultured embryos so that they can be taken into consideration as possible target organs for the hormone. The present ultrastructural study on the metanephros of cultured embryos is directed towards this objective.

In addition we have conducted determinations of the concentration of cyclic AMP in metanephros from chick embryos stimulated with high doses of parathyroid hormone. It has been shown that the cellular effects of this hormone are dependent on an activation of the adenylate cyclase-cyclic AMP messenger system (Aurbach et al., 1972) and the above mentioned determinations were conducted with the hope of obtaining additional information on the possibility of the embryonic kidney being a target tissue for the hormone.

Material and Methods

White Leghorn eggs were incubated in a forced-draught incubator for 3 days. After their surfaces had been cleared with a solution of benzalconium chloride (Zephiran, Winthrop, 1:750) they were opened and their contents were cultured (Auerbach et al., 1974) in 20 × 100 mm plastic Petri dishes (Falcon, No. 3003). Dishes were then covered and incubated at 37°C in an atmosphere of air saturated with water vapor for periods ranging between 8 and 11 days. After incubation, embryos were examined and their developmental stage was determined according to Hamburger and Hamilton (1951). Cultured as well as control embryos of 10 to 13 days incubation were dissected and the metanephros cut into pieces and fixed in cold half-strength Karnovsky's fixative (Karnovsky, 1965) for 6 h. Tissues were then washed in cacodylate buffer at pH 7.4 with 0.2 M sucrose, post-fixed in 1% osmium tetroxide for 2 h, dehydrated with ethyl alcohol and embedded in Araldite. One μm sections were stained with toluidine blue for