A new model of glucocorticoid-induced metanephric maldevelopment[1]

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Summary. A new experimental model of glucocortocoid-induced tubular cyst formation has been developed in metanephric organ culture. The addition of cortisol (1.4×10^{-5} M) to chemically defined serum-free culture medium produces cystic changes during in vitro nephrogenesis. The model isolates the role of glucocorticoids in experimental cyst formation.

Renal cystic changes have been experimentally induced in different animal species with a variety of glucocorticoids[3]. The independent role of glucocorticoids in mediating the observed cystic changes has remained controversial, since alterations have been produced only in combination with severe potassium depletion, and cyst formation has been prevented by potassium chloride supplementation[4,5]. We have therefore studied the isolated role of glucocorticoids in inducing metanephric cystic development in our recently described serum-free mouse metanephric organ culture system[6]. Through addition of glucocorticoids to completely characterized, serum-free medium, we have developed an experimental model in which renal polycystic changes occur during in vitro nephrogenesis with the maintenance of a constant potassium and chloride environment.

Materials and methods. Our method of whole metanephric organ culture has been described in detail[6]. Pregnant Swiss-Webster albino mice were sacrificed by cervical dislocation at 13 ± 0.4 days gestation. Under aseptic conditions fetal metanephric tissue was microdissected from embryos and transferred to a 0.8 µm Millipore filter sitting atop a Trowell double-welled organ culture assembly. Culture medium for control tissue consisted of equal volumes of Dulbecco's modified essential medium and Ham's F-12 medium supplemented with transferrin (5.7×10^{-7} M), prostaglandin E_1 (7.1×10^{-8} M), insulin (8.7×10^{-8} M), triiodothyronine (2×10^{-9} M), and Na_2SeO_3 (7.6×10^{-9} M). The cystic model was produced by the addition of glucocorticoids to the control medium. Both cortisol, in concentrations ranging from 7×10^{-6} M to 5.6×10^{-5} M, and dexamethasone, in concentrations ranging from 2×10^{-7} M to 1×10^{-5} M, produced dose-related progressive cystic tubular changes in explants when added to control medium. All glucocorticoids were obtained in the crystalline form (Sigma Chemical, St. Louis, MO), solubilized in sterile 95% ethanol, and passed through a 0.22 µm cellulose ester membrane filter. Glucocorticoids were added to culture medium at the initiation of the organ culture procedure, and maintained at appropriate concentrations throughout the 6-day culture period. The concentrations of potassium (3.9 mmoles/l) and chloride (124 mmoles/l) were maintained constant in both control and cystic medium, and all tissue was incubated at 36 ± 0.2 °C and 95% humidity in a mixed air -5% CO_2 environment. Culture medium was changed every 48 h and tissue was sampled daily for histological analysis and viability measurements as previously described[6].

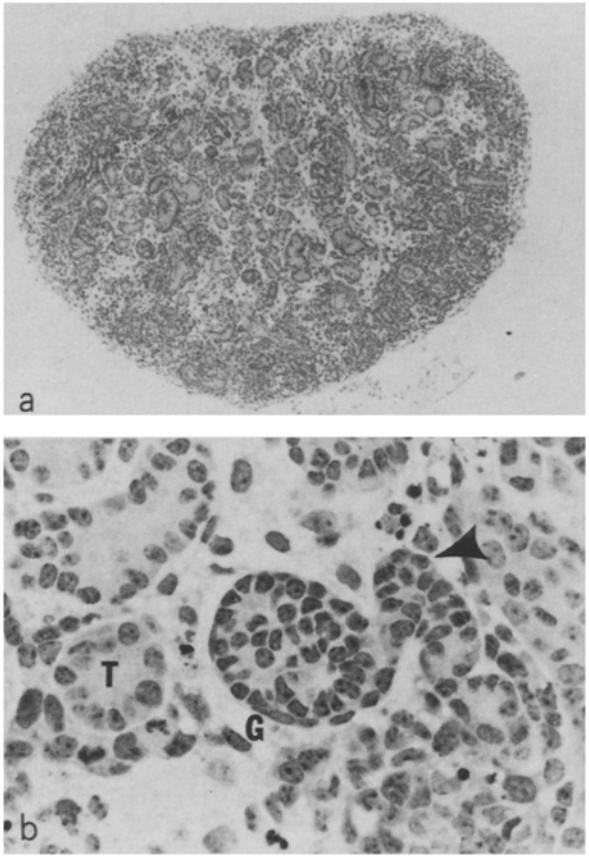

Figure 1. Control tissue following 144 h of incubation. *a* The intact metanephric explant exhibits a panorama of active nephrogenesis. Hematoxylin × 54. *b* Mature tubules (T) are present, and glomeruli composed entirely of epithelial elements (G) have developed in continuity with segments of proximal tubules (arrowhead). Hematoxylin × 420.

Results. Although cystic changes were produced in developing explants by both cortisol and dexamethasone in varied concentrations, the best balance of metanephric differentiation, tissue viability, and cystic alterations was produced by cortisol at a concentration of 1.4×10^{-5} M. This glucocorticoid preparation was therefore used to produce cystic alterations in all studies designed to define the characteristics of the cystic metanephric model.

Following 144 h of culture, control tissue showed advanced organotypic development including the formation of mature tubules and in vitro glomeruli composed entirely of epithelial elements (fig. 1a and 1b). In contrast, hydrocortisone-treated tissue following the same period of in vitro incubation showed marked tubular cystic maldevelopment against a background of normal nephrogenesis (fig. 2a). Cyst walls showed a flattened epithelial lining with vacuolar change, and in some areas tubular supporting structures had retracted behind the base of cyst walls (fig. 2b). Cellular viability of both treatment and control groups remained above 85% during the 6-day culture period.

Discussion. Injection of glucocorticoids has produced experimental renal cystic changes in newborn and adult rabbits,[4,7] newborn hamsters[8], and newborn rats[5]. In such in vivo studies it has been impossible to separate the specific effects of glucocorticoids on nephrogenesis from the secondary effects of metabolic abnormalities induced in treated animals. The independent role of glucocorticoids in inducing cystic changes has been questioned, and concomitant potassium or chloride depletion have been considered factors necessary for drug effect[3]. In the current study both cortisol and dexamethasone produced cystic metanephric maldevelopment in a highly controlled organ culture system in which environmental potassium and chloride were kept constant. This attests to the unique role of glucocorticoids in experimental cyst formation.

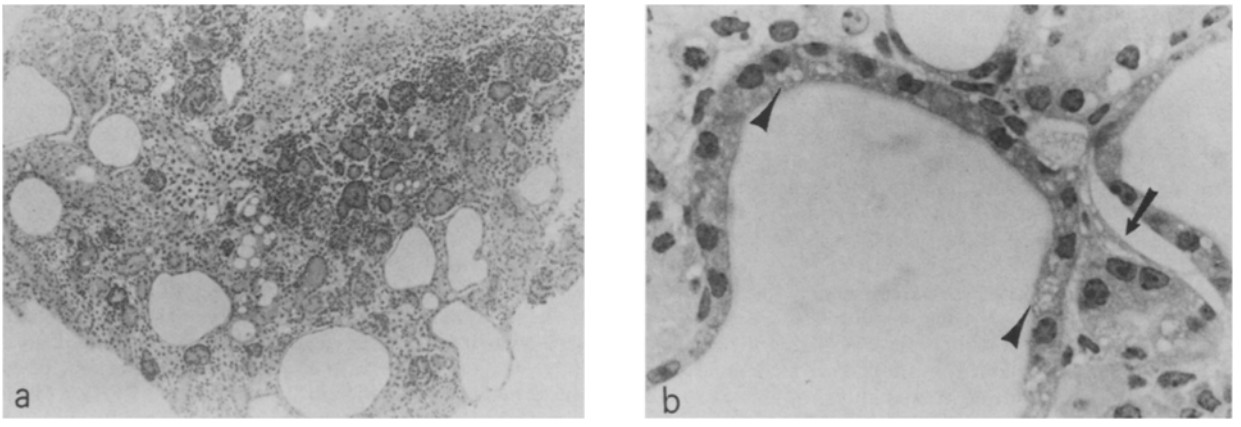

Figure 2. Tissue treated with cortisol (1.4×10^{-5} M) following 144 h of incubation. *a* Cystic tubular changes are present amidst a background of tubular and glomerular elements. Hematoxylin × 54. *b* Flattened epithelial cells of cyst walls show vacuolar changes (arrowheads), and tubular supporting wall structures are retracted behind the bases of cysts (arrow). Hematoxylin × 420.

The precise mechanism by which glucocorticoids induce cystic changes has not been determined. In the organ culture model, cortisol induces cystic abnormalities in developing renal tissue without vascularization, glomerular filtration, or urine formation. Since nephron obstruction and increased intratubular hydrostatic pressure cannot occur in the nonperfused system, the model isolates the role of drug-induced alterations in epithelial cell and tubular supporting wall structure and function for further study. The morphological features of tubular cell flattening, intracellular vacuolization, and retraction of cyst wall supporting tissue suggest diffuse drug effects on intracellular metabolism and extracellular matrix formation with resultant increased tubular wall compliance. This is consistent with known glucocorticoid effects on protein synthesis and extracellular matrix production[9,10]. Studies are currently underway to define the molecular and biochemical events which may mediate glucocorticoid-induced cystic changes in this model.

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Enhancement of muscle regeneration by bone marrow cells in the monkey

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Summary. Transplantation of muscle minces with and without autogenous bone marrow cells was performed in the monkey. The addition of bone marrow cells markedly enhanced muscle regeneration. The findings suggest a possible clinical application of the technique.

Muscle growth and regeneration in health and disease have been the subject of extensive recent research[2–5]. However, results of clinical trials with therapeutic muscle transplantations have generally been unsuccessful because of poor myogenic regeneration, fibrosis of the transplant and inadequate restoration of function[2,3].

Our previous work with muscle cultures[6,7] and transplantation of muscle minces in rats[8] has shown that marked enhancement of myogenesis occurs when autogenous bone marrow cells are added to muscle minces. The procedure was also found to suppress bacterial infection in both the transplant and in culture. The present work was undertaken to see if the effect of adding autogenous bone marrow cells to muscle mince transplants in a young adult monkey would, as in rats, enhance myogenesis.

Material and methods. 2 separate sets of experiments were performed with a 10-month interval between, on an adult baboon weighing 10 kg. The monkey was anesthetized with nembutal 4 ml and pentothal 3 ml i.m. Surgery was performed under strictly sterile conditions. 2.0 ml of bone marrow was aspirated from the sternum using a 14 gauge needle and mixed with