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Human Embryonic Kidneys in Organ Culture: Abnormalities of Development Induced by Decreased Potassium

Abstract. *Human embryonic kidneys of 5 to 12 weeks of gestation were grown in organ culture. Potassium concentrations of 3 to 6 milliequivalents per liter produced decreased ureteral bud branching, failure of nephron induction, and occasional cystic dilatations of the ureteral bud. Normal development of the kidney occurred at potassium concentrations of 6.5 to 10 milliequivalents per liter. These studies confirm the importance of relative stability of the potassium concentration in the development of the embryonic kidney.*

It is important to determine the relevance of experimental renal teratogenesis in animals to human disease. Studies in human embryonic or fetal tissue would be the most reliable index of mechanisms of growth abnormalities in man.

Perey *et al.* (*1*) produced a disease similar to human polycystic kidney disease by injecting high doses of steroids into newborn rats. Their data suggested that hypopotassemia, induced by the mineralocorticoids, was responsible for the morphologic changes. Crocker and Vernier (*2*), using 1000 whole organ cultures of fetal mouse kidney, showed that alteration of the potassium concentration was one of the likely causes of this renal maldevelopment. They emphasized the necessity of constant maintenance of potassium concentration for normal renal differentiation in the early stages of development. Stewart and Welt (*3*) and Serrano *et al.* (*4*) showed that when the maternal serum potassium concentration in the pregnant dog was lowered by dialysis to the range of severe hypokalemia, potassium concentration in the fetus was not affected. Similarly, Dancis and Springer (*5*) showed that high plasma potassium in fetal rats was maintained when the mothers were fed potassium-deficient diets. In the crucial early stages of renal differentiation, the embryonic or fetal plasma potassium may be kept stable by a pump, probably at the placental level.

We report the development of a whole organ culture model for human kidney, in which we studied the teratogenic effects of potassium concentrations of 3 to 6 meq/liter. Oxygen concentration and acid-base stability must be closely regulated, because changes in nephron development patterns may be caused by these factors (*6*). Our observations emphasize the requirement of high embryonic serum potassium concentrations for human renal development.

Whole human kidney pairs from 75 embryos 5 to 12 weeks of gestation were obtained by vacuum curettage. After curettage, the material in the vacuum apparatus was washed rapidly in Hanks balanced salt solution through a multiple mesh screen system. This removed most conception products other than the embryo. Portions of embryos or whole embryos of 5 to 12 weeks of gestation were usually obtained. The kidneys were removed rapidly with cataract knives while the embryo was viewed under a Zeiss stereomicroscope, and were placed in Hanks balanced salt solution.

Kidneys were placed on a Nuclepore membrane filter supported by stainless steel wire mesh in a Falcon plastic organ culture dish, or were grown submerged on a Nuclepore filter in the dish. All cultures were oscillated (2 min^{-1}) on a Bellco rocker to improve circulation of the tissue culture medium. Human kidney of this size and gestational age was chosen because (i) small kidneys are easy to handle; (ii) their use minimizes the problem of central necrosis, which is apparently caused by hypoxia during prolonged organ culture; and (iii) human metanephric kidneys begin differentiation at 35 days of embryonic life and, as judged from previous animal studies in this laboratory (*2*), the first weeks of renal embryonic development would be the period of maximum sensitivity to potassium concentration variations.

The cultures were incubated in a water-jacketed incubator at 37°C in 80 or 95 percent oxygen with 5 percent carbon dioxide. Oxygen was monitored with a Beckman model D oxygen analyzer. Media were changed every 48 hours. Medium 199, prepared virtually free of potassium (0.01 meq/liter) with Hanks base (*7*), was supplemented with extract of 9-day chick embryos (*8*), 4 percent; horse serum, 10 percent; penicillin, 200 unit/ml; streptomycin (*7*), 200 mg/ml; and Mycostatin (*7*), 100 units/ml. Electrolyte concentration of the final medium was measured by flame photometry after a known quantity of potassium solution was added. The *p*H of the medium was controlled by titration with 7.5 percent sodium bicarbonate solution. Cultures were grown for 2 to 5 days and kidneys were then processed by standard histological fixation techniques. Normal human embryonic kidneys at similar gestation age were also studied and compared with experimental organs to be certain of organ growth and development. Of each kidney pair, the control organ was grown in medium with a potassium concentration of 6.5 to 10 meq/liter, while the other was grown with potassium of 3 to 6 meq/liter.

In 38 embryonic kidneys grown with potassium concentrations of less than 6 meq/liter, the following defects were seen (Fig. 1B): (i) a decreased number of branches of the ureteral bud, (ii) failure of nephron induction at the site of branching, and (iii) occasional dilatation of the ureteral bud. The paired control embryonic kidneys grown in media containing 6.5 to 10 meq/liter grew normally and were free of the defects seen in those grown at lower potassium concentrations. The renal pelvis and calyces were normal if kidneys of later gestational age were grown in culture. Severe signs of previous anoxia or damage (thought to be due to the vacuum extraction procedure) made 37 pairs of kidneys unsuitable for evaluation. The teratogenic

effect of low potassium concentration could be detected after 48 hours of culture but became more extensive as culture was prolonged.

This study demonstrates major developmental defects of human embryonic kidneys grown in vitro at potassium concentrations relatively low for the embryonic environment. Induction of the metanephros begins at 35 days of gestation when the ureteral bud, growing up from the bladder area, acts as inducer for an elongated clump of cells along the posterior wall of the embryo. The classical work of Grobstein and his colleagues (*9*) concerning the complex aspects of ureteral bud induction of metanephrogenic tissue into the proximal nephron in vitro has stimulated much research on defects of induction. However, the complexities of induction and differentiation are still incompletely understood (*10*).

That changes in external potassium concentration could affect ureteral and metanephrogenic cell metabolism is not without precedent. MacDonald *et al.* (*11*) noted changes in transmembrane secretory potential and other intracellular energy and enzyme systems when external potassium was augmented. As suggested by Petersen (*12*) for secretory cells and Casteels *et al.* (*13*) for muscle membrane, removal of external potassium causes a decrease in potassium conductance of the cell membrane. Naslund and Hultin (*14*) showed that mammalian ribosomes, like those of marine invertebrates (*15*), become unstable in the absence of potassium ion. The specificity of the stabilization by the potassium ion is fairly high; of the commonly used monovalent ions, only ammonium and rubidium had an effect comparable to that of potassium.

Low concentrations of potassium in the medium of organ cultures appear to influence all phases of development of the ureteral bud beyond the initial branching and formation of the pelvis and calyces. The mechanism (or mechanisms) for maintaining a high concentration of potassium in the embryo, the role of the ion in fetal development, the drugs or chemicals capable of affecting the mechanisms, and the possible role of hypopotassemia as a cause of human kidney maldevelopment demand further study.

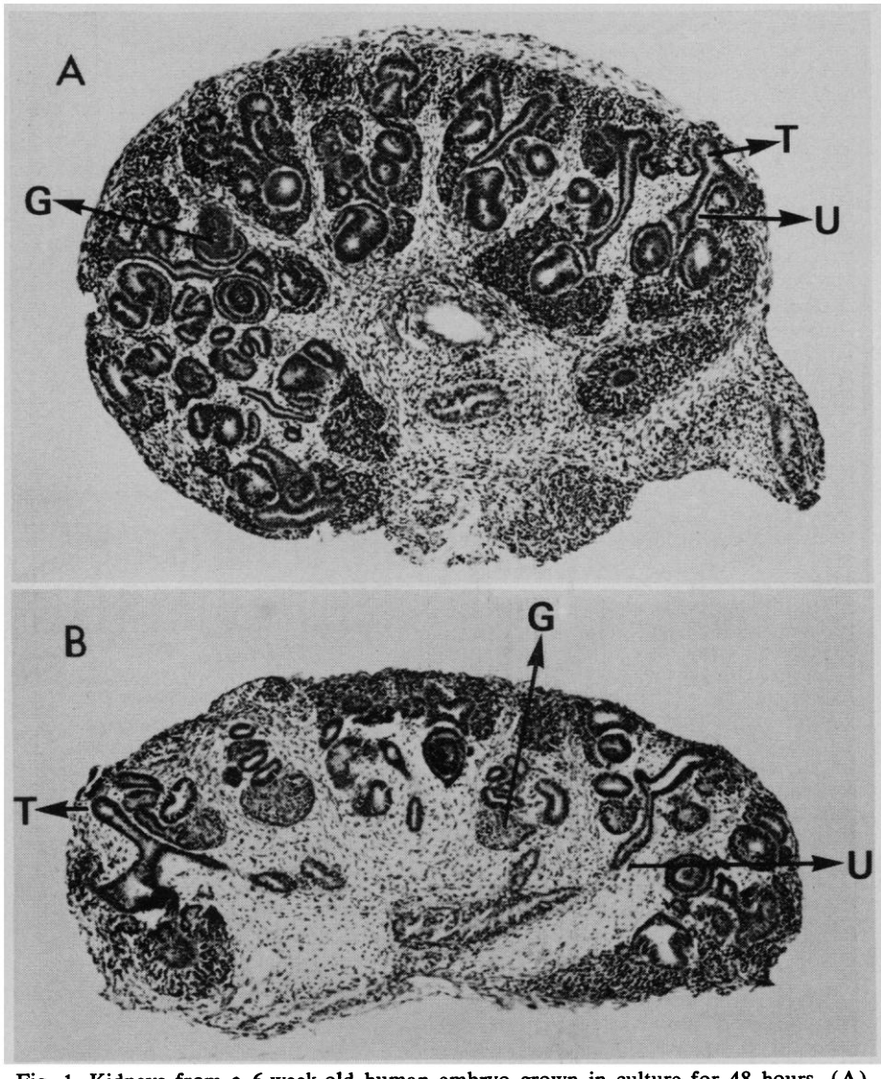

Fig. 1. Kidneys from a 6-week-old human embryo grown in culture for 48 hours. (A) The concentration of potassium in the medium was 8.5 meq/liter. The ureteral buds (*U*) branch repeatedly; terminal branches (*T*) induce nephrons normally; and there are many nephrons with glomeruli (*G*) in different stages of development. (B) The concentration of potassium in the medium was 4.9 meq/liter. Ureteral buds (*U*) branch less frequently, and there are fewer terminal branches (*T*), which do not appear to be inducing glomeruli (*G*). The number of developing nephrons is decreased compared with (A), and there are dilated areas of ureteral bud. (× 160)

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