speciation process, to account for the present quantitative differences.

To explain the significantly different Feulgen-DNA contents per unit length of chromosome which we observed in the three species of planarians, it may be hypothesized either 1) that metaphase condensation and coiling of DNA is actually different in the species examined; or 2) that there are differences of the M-phase length during the cell cycle, so that colchicine treatment always for the same length of time (as we carefully used) induced a different chromosome condensation at the moment of slide preparation. In the present state of the research, neither hypothesis can be conclusively ruled out; however, both explanations point to the existence of species-specific cytologic peculiarities within the genus *Dugesia*, besides the genome size differences observed.

In a previous paper[7], a fairly good correspondence was found between karyometric and microdensitometric values for the relative sizes of single chromosomes of the E and F biotypes of *D. lugubris*; however, some discrepancies between the results obtained with the two methods were noticed for the smallest elements of both karyotypes, so that the possibility was underlined that, in these chromosomes, DNA could be differently packed. That evidence and the findings of this paper show that no direct correlation may exist between Feulgen-DNA amount and linear karyotype length, and suggest that particular attention must be paid when comparing genome size estimates obtained by these two methods.

From a methodological standpoint, once more it is apparent that for the purpose of genome size determination the cytochemical approach is definitely preferable, both because it is more directly related to the actual amount of genetic material (which can be measured also on interphase nuclei) and because it is much less affected than the karyometric methods by the degree of chromatin condensation (or DNA coiling).

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Regression of genetically determined polycystic kidney disease in murine organ culture

E. D. Avner, W. E. Sweeney Jr, N. P. Piesco and D. Ellis

Division of Nephrology, Children's Hospital of Pittsburgh, 125 DeSoto Street, Pittsburgh (Pennsylvania 15213, USA), 28 March 1985

Summary. Cystic kidneys from the mutant CPK strain of C57BL/6J mice were cultured in serum-free organ culture. During 120 h of incubation in chemically-defined medium, CPK cystic tubular changes underwent complete regression. Environmental factors regulate the expression of genetically determined polycystic kidney disease in this model.
Key words. Polycystic kidney disease; organ culture.

Genetically determined polycystic kidney diseases (PKD) cause significant morbidity and mortality in both children and adults[1,2]. Despite the clinical importance of such disease states, the basic pathogenetic mechanisms operative in human renal cyst formation have not been clearly identified. Further, the factors responsible for the wide variation in the clinical expression of PKD remain unknown. Recent studies of toxin-induced renal cystic disease in rats have demonstrated that renal cyst formation may be totally reversible under certain experimental conditions, and that environmental factors may modulate the progression of cystic tubular changes[3,4]. Such data raise the possibility that the expression of genetically determined renal cyst formation may be regulated by the biochemical and microbial environment in which such kidneys develop. We therefore studied the development of autosomal recessive polycystic disease in kidneys from the mutant. CPK strain of C57BL/6J mice[5,6] under the highly controlled conditions of our previously described serum-free metanephric organ culture system[7,8]. The organ culture system permits organotypic growth and differentiation of renal tissue in chemically defined medium without perfusion, filtration, or urine formation. It was thus possible to experimentally isolate the process of genetically determined renal cystic maldevelopment from flow-related phenomena or the presence of cyst-promoting substances in CPK serum or urine.

Materials and methods. Our basic method of intact metanephric organ culture has been described in detail[7,8]. In the current study, newborns from matings of control C57BL/6J mice as well as newborns from matings of heterozygotes for the CPK trait were sacrificed by decapitation. Paired kidneys of each newborn were aseptically removed and one was placed in organ culture medium at 4 °C while the other was processed for light microscopy. The organ culture medium consisted of equal volumes of Dulbecco's modified essential medium and Ham's F-12 medium supplemented with selenium, 6.8×10^{-9} M; insulin, 8.3×10^{-7} M; triiodothyronine, 2×10^{-9} M; transferrin, 6.2×10^{-8} M; and prostaglandin E_1, 7.1×10^{-8} M. For organ culture, the kidney was cut into explants of 100–120 µm thickness utilizing a custom designed microslicer. Explants were trimmed and transferred onto a 0.8-µm Millipore filter sitting atop a Trowell-type double-welled organ culture assembly. The assemblies were incubated at 36 ± 0.5 °C and 95% humidity in the mixed air-5% CO_2 environment of a water-jacketed incubator. Culture medium was replenished every 24 h, and tissue was sampled daily for histological analysis and viability measurements as previously described[7,8].

For each kidney explanted into organ culture, its paired mate was processed for light microscopy and served as histological control. This assured clear identification of cystic kidneys from

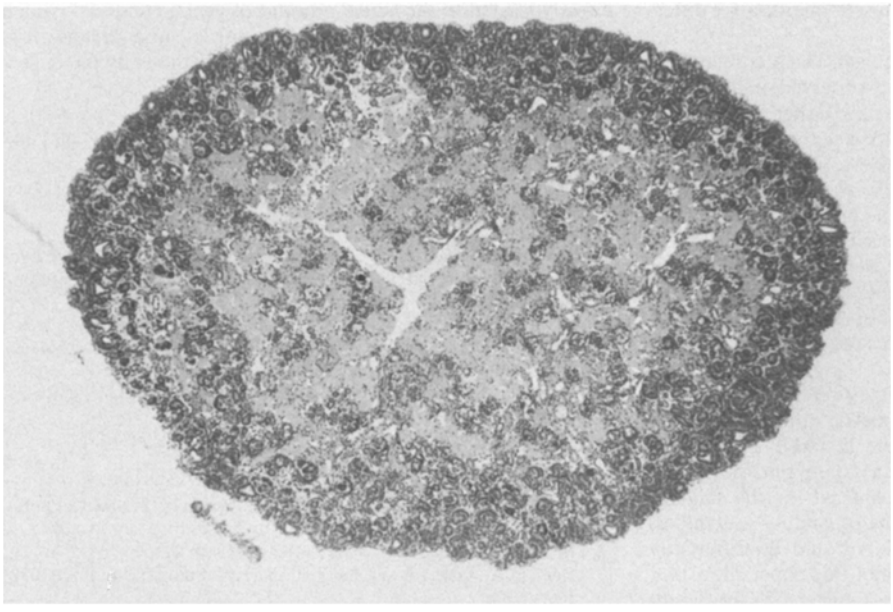

Figure 1A. Control explant prior to culture. An array of developing tubular and glomerular structures extend with increasing density from medulla to peripheral cortex. (Hematoxylin, ×140.)

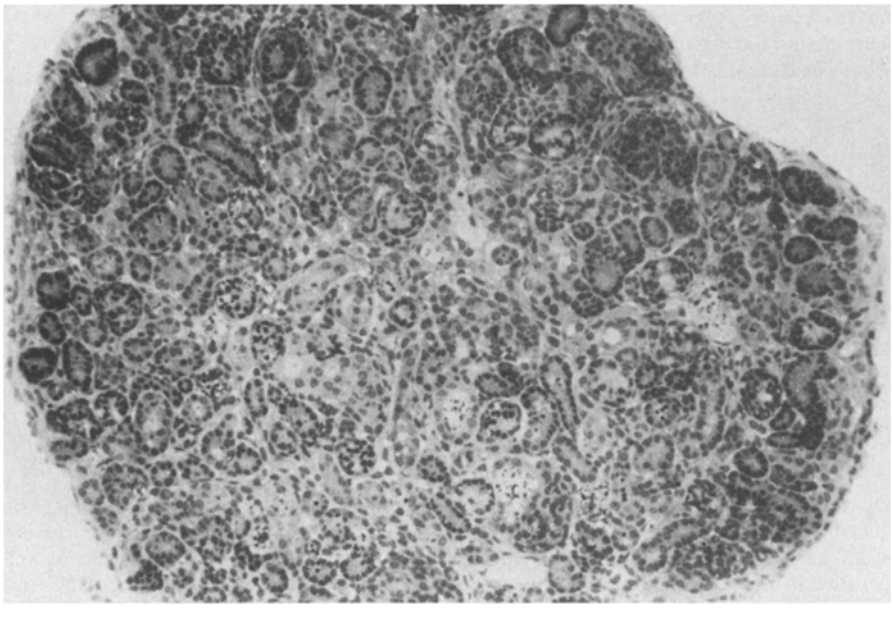

Figure 1B. Control explant following 120 h of organ culture incubation. Organotypic tubules and round, glomerular-like bodies are present uniformly throughout the tissue. No evidence of tissue injury or necrosis is present. (Hematoxylin, × 340.)

offspring homozygous for the CPK trait, since all such affected kidneys show microscopic evidence of tubular cyst formation at this stage[5,6].

Results. At the initiation of the culture period, control explants showed the normal panorama of developing tubular and glomerular structures (fig. 1A). A clear gradient of increasing nephrogenesis was present from the central medulla to the peripheral cortex of the tissue. During 5 days of organ culture incubation, control explants demonstrated continuing nephrogenesis without evidence of necrosis. By 120 h of culture, explants consisted of tubular elements and round, glomerular-like structures which extended homogeneously from the center to the periphery of the tissue (fig. 1B). At the time of explantation into organ culture, explants from cystic CPK kidneys showed prominent tubular dilatation and early cyst formation against a background of normal nephrogenesis (fig. 2A). As previously noted, such changes were largely localized to proximal tubular elements[6]. During 5 days of organ culture incubation in defined, serum-free medium, a gradual reversal of cystic tubular changes occurred. By 120 h of culture, cysts had completely regressed, and explants exhibited a variety of tubular elements and organ culture glomeruli which were indistinguishable from those seen in cultured controls (fig. 2B). Cellular viability of both control and CPK explants remained consistently above 85% during the 5-day culture period.

Discussion. Investigations into the pathogenesis of genetically determined renal development have been limited by a paucity of suitable, naturally-occurring animal models of congenital PKD. In 1977 a spontaneous mutation in the C57BL/6J murine line produced a renal cystic disease which was subsequently transmitted as an autosomal recessive trait. Although appearing normal at birth, affected CPK animals show abdominal protuberance secondary to their enlarging cystic kidneys by 10–13 days of postnatal age and die in renal failure 3–4 weeks postna-

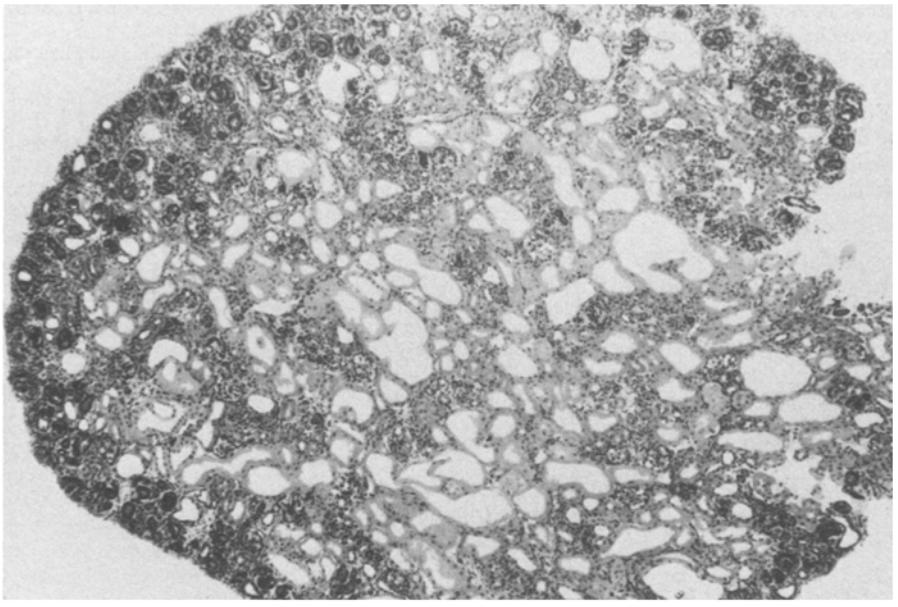

Figure 2A. CPK explant prior to culture. Extensive tubular changes ranging from mild dilatation (primarily cortical) to frank cystic malformation (primarily medullary) are present amid normal nephron elements. (Hematoxylin, ×140.)

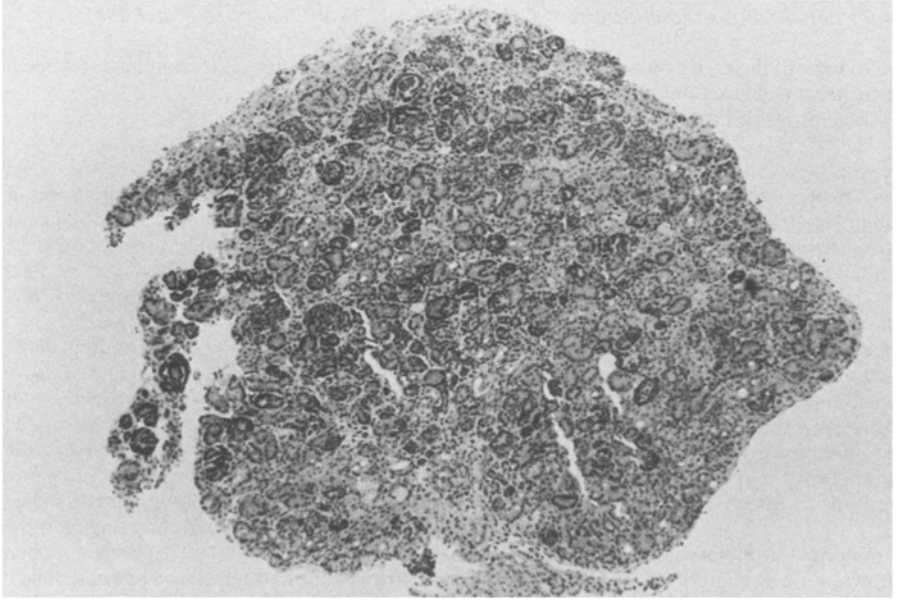

Figure 2B. CPK explant following 120 h of organ culture incubation. A complete regression of cystic tubular changes has occurred. The explant exhibits tubules and glomerular-like structures which cannot be distinguished from those of cultured control tissue (compare with figure 1B). (Hematoxylin, ×140.)

tally[5,6]. In sharing a number of pathological as well as clinical features with the two major forms of human congenital PKD, the CPK mutant provides the opportunity to study the pathophysiology of cyst formation in a suitable animal model of genetically determined renal cystic disease. The specific application of established organ culture methodology to the study of CPK kidneys permits complete experimental control of the biochemical environment of cyst development. The utilization of such methods therefore experimentally separates a genetically programmed developmental defect from the environment which may influence such a defect's expression.

The current study emphasizes the reversible nature of early tubular cystic changes in CPK kidneys and suggests that genetically programmed alterations in tubular cell structure and function are conditioned by the environment in which such cells develop. Such findings extend the recent observations that acquired renal cystic disease induced in rats by various toxins may undergo complete resolution if toxin is withdrawn or the animals are reared in a strictly aseptic environment[3,4]. Our data do not clearly establish whether the reversal of CPK cyst formation in the nonperfused organ culture system was promoted by the removal of tissue from a toxic or infected environment, the dissipation of possible cyst-promoting hydrostatic forces, or the presence in the culture medium of specific inhibitors of the cystic process. Previous investigations from our laboratory have demonstrated that cystic tubular malformation may be experimentally produced under aseptic organ culture conditions without filtration or changes in the basal medium of the system[9–11]. Thus, the simple elimination of filtration related hydrostatic forces or the presence of cyst-inhibiting factors in the culture medium would not appear to be likely explanations for the cyst regression seen in the current studies. The possibility that the observed reversal of cystic tubulogenesis was promoted by removal of CPK tissue from an in vivo cyst-promoting microenvironment is

supported by the preliminary report that homozygous CPK mice have serum corticosterone levels which are eight times higher than those of age-matched controls[12].
This finding is of particular importance in light of the proven cyst-inducing effects of various glucocorticoids[10,11,13,14]. Studies are currently underway to further define the environmental factors which may promote, as well as prevent, the development of genetically programmed cystic tubular changes in the CPK model.

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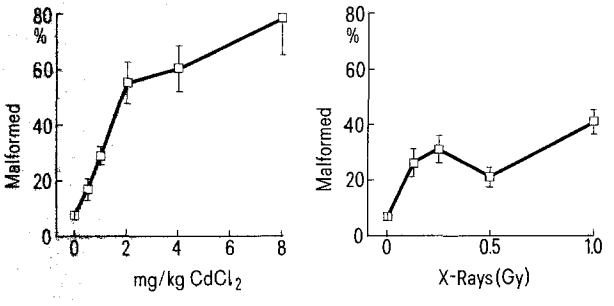
Teratogenic interactions between cadmium and radiation in mice

C. Michel and H. Fritz-Niggli

Strahlenbiologisches Institut der Universität Zürich, August-Forel-Strasse 7, CH-8008 Zürich (Switzerland), 13 March 1985

Summary. Mouse embryos were exposed to various doses of cadmium and/or X-rays on day 8 of gestation. The combined treatment exerted an antagonistic effect regarding the teratogenic action of the two agents.
Key words. Embryonic development; mice; teratogenic interaction; cadmium; X-irradiation.

The contamination of the environment with heavy metals, and their potential toxicity, have received increasing interest. Concern about health effects of cadmium mainly relates to the carcinogenic, teratogenic and mutagenic action of this metal. So far, there is no conclusive evidence that cadmium produces these effects in humans[1,2].
Regarding cadmium-induced embryotoxicity, animal experiments demonstrate a broad spectrum of developmental anomalies, depending on dose, gestational age, application and genetic factors[3–8]. It is evident that several chemicals and environmental factors modify the teratogenicity of heavy metals[9]. There are indications that additive or synergistic relationships exist between in vivo application of Cd^{2+} and radiation[10]. Experiments in vitro with preimplantation mouse embryos revealed an additive behavior of the two agents[11].
In our previous studies, the combined treatment of mouse embryos with radiation and drugs has shown various degrees of potentiation[12,13]. The objectives of the present work are to determine the embryotoxic effects of cadmium and possible interactions in vivo with X-rays during a highly sensitive stage of development in mice.
Materials and methods. Virgin female NMRI-mice, aged 10–12 weeks, were mated overnight and examined for a copulation plug the next morning (day 0 of gestation). On day 8, pregnant animals were whole-body exposed to 0.125, 0.25, 0.5, and 1.0 Gy of 200 kV X-rays (12 mA, 1 mm Al + 0.5 mm Cu filtration, 1. HVL = 0.93 mm Cu, dose rate 0.465 Gy/min). Five groups of mice were injected i.p. with $CdCl_2$ on day 8 (0.5–8.0 mg/kg b.wt). Control animals were injected with 1 ml of physiological saline. In combined treatment 2 mg/kg $CdCl_2$ was injected 30 or 60 min before or immediately after irradiation. The experiments were made in series of replicates, each with a group of 5–8 females. On day 13 of gestation the fetal mortality, growth retardation and malformations were evaluated. The data were analyzed using the Mann-Whitney ranking test.
Results. Concerning teratogenic effects of cadmium, a linear dose-effect curve was found up to the dose of 2 mg/kg (fig.). Qualitatively, microphthalmia dominated at lower Cd doses whereas high frequencies of exencephaly occurred with doses of 2 and more mg/kg. At the highest dose (8 mg/kg), 55% of the implantations were dead, i.e. embryolethality prevails over the induction of malformations. Up to 4 mg $CdCl_2$/kg the fetal mortality rates varied between 6% and 9.4% compared to 10% in the control (NaCl) group ($p > 0.05$).
The dose effect relationship for radiation-induced teratogenicity between 0.125 and 1.0 Gy is not linear (fig.). In qualitative respects, exposure to 1 Gy resulted in a significant increase of exencephaly compared to lower doses (table). No increase in the rates of lethality and growth retardation were observed in the irradiation groups in comparison to control mice.
So far, combined application of cadmium (2 mg/kg) and 0.5 or 1.0 Gy has been tested (table). The most interesting finding is the reduced rate of malformations in the co-insult experiments when Cd was applied 30 or 60 min before irradiation. The antagonistic relation in the teratogenic activity was less pronounced when cadmium was given 60 min before 0.5 Gy. The decreased general malformation rate is mainly due to the generally lower incidence of exencephaly (8% and 16% compared to 35% in the Cd



Teratogenic effects of cadmium alone (left) and X-rays alone in 13-day-old mouse fetuses after treatment on day 8 of gestation.