antibody and clone 8 Fab fragments was nearly identical.

With such a large quantity of antibody present (Fig. 1A) it is important to consider why biological activity remains at all. Smaller quantities of polyclonal antibody are capable of completely abolishing biological activity in similar experiments (*17*), perhaps by precipitating β-NGF through multiple determinants. Since the Fab fragment of clone 8 antibody cannot remove NGF from soluble phase dynamic equilibrium, the addition of target cell receptors to the system would be expected to shift the equilibrium away from the antigen-antibody interaction. This is thermodynamically favorable: the high-affinity binding constant of the NGF-target cell interaction is on the order of 10^{11} liter per mole (*18*), whereas average association constants of antigen-antibody interactions are generally 10^2- to 10^4-fold lower. These results are consistent with an antibody-NGF affinity constant on the order of 10^8 liter per mole.

It is possible that the α or γ subunit could mask or sterically inhibit binding of clone 8 antibody to its determinant while not interfering with the receptor binding of β-NGF. This possibility has been excluded because the α and γ subunits do not inhibit the binding of clone 8 antibody to β-NGF in the ELISA, even at concentrations of 0.5 mg/ml. The clone 8 antibody does not bind to the α or the γ subunit because the ELISA gave negative results with these antigens.

There is evidence that the active site of β-NGF is well conserved evolutionarily (*5*), whereas other determinants may have diverged rapidly (*2*). This monoclonal antibody reacted with mouse β-NGF, snake venom (*Naja naja*) β-NGF, and human β-NGF in the ELISA, indicating that the antigenic determinant recognized has been strictly conserved during evolution. This conflicts with other studies (*19*) which failed to demonstrate cross-reactivity between mouse β-NGF and human β-NGF.

The production of a monoclonal antibody that is directed toward a determinant at or near the active site of the β subunit of NGF may be extremely fortunate, since polyclonal antiserums appear to be directed mainly toward less specific antigenic determinants (*5*). The availability of high-affinity monoclonal antibodies that specifically inhibit the biological activity of mouse β-NGF will permit studies on the similarity of active sites between mouse and other species, the distribution of NGF among various tissues, the interaction between β-NGF and its receptor, and the mechanism of action of the hormone factor. It will also be of interest to define the structural determinants to which various monoclonal antibodies bind on each of the subunits and, hence, the nature of the interaction within the 7*S* NGF complex.

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Transporting Renal Epithelium: Culture in Hormonally Defined Serum-Free Medium

Abstract. *A hormonally defined medium was used to isolate a homogeneous epithelioid cell population from canine kidney. Monolayers of these cells form domes, an indication of active ion transport, and this process is inhibited by ouabain. This technique allows the isolation of primary cultures of renal epithelial cells, free of fibroblasts, for the characterization of biochemical and physiological properties related to renal function.*

Models have been used to study the biochemical events associated with the hormonal regulation of ion transport by the kidney. These models include the urinary bladders of amphibia and reptilia and, most recently, cells in tissue culture (*1*). Active ion transport occurs in a number of different epithelioid cell cultures (*2–10*) and is characterized by the formation of structures designated as hemicysts or domes. These domes represent areas of localized detachment of the monolayer caused by the accumulation of fluid between the basolateral membrane and the culture dish (*11*) as the result of active ion transport from the apical to the basolateral surfaces of the cells. Dome formation is inhibited by agents that block transepithelial sodium transport, such as ouabain (*6, 12, 13*), and is thus presumed to be associated with transport processes related to normal renal function.

Normal cells grown in a tissue culture environment, which provides control of the external milieu and cell homogeneity, would be useful for the study of biochemical and physiological mechanisms related to kidney function and their hormonal regulation. Attempts at the isolation of normal epithelioid cell lines from the kidney have been generally unsuccessful, however, because of persistent problems with the elimination of contaminating fibroblastic cells, the isolation of a specific cell type, and the definition of a medium in which specific cells can proliferate while maintaining differentiated properties. A number of investigators have therefore used continuous epithelioid kidney lines (for example, MDCK), to investigate biochemical mechanisms related to renal function. All of these continuous cell lines exhibit aberrant biochemical or physiological properties when compared to cells that have not undergone transformation. Therefore, a primary kidney cell culture system should be of significantly greater value in the study of transport mechanisms. We have used a hormonally defined medium (*14, 15*) to isolate and grow, free of fibroblasts, a morphologically uniform epithelioid canine kidney cell population that forms domes.

Seven healthy mongrel dogs, four male and three female (4.3 ± 0.9 years old, weighing 27.5 ± 0.9 kg), were

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anesthetized with pentobarbital (28 mg/kg), and the kidneys were removed. A piece of outer cortex (not more than 3 mm below the renal capsule) was aseptically excised from each kidney. The tissue was dissociated (100,000 viable cells per milligram of cortex, wet weight), and at least four culture flasks (25 cm^2) were started with 2×10^5 cells per flask in both hormonally defined medium and in serum-supplemented maintenance medium (*14*).

In the maintenance medium a large number of cells became attached after dissociation and plating, and a substantial amount of cell growth was observed in all cultures. These primary cultures grew at a greater rate (doubling time, 23.0 hours) than cells grown in hormonally defined medium (doubling time, 40.6 hours) and appeared to contain one cell type, an epithelioid cell (cell surface, 1050 ± 58 μm^2) without any unusual morphological traits. In these cultures, the epithelial cells were never observed to form a tight-looking (cobblestone) monolayer, nor were domes ever observed. After the first passage (80 percent confluency), with a split ratio of 1:3, epithelioid cell growth declined. The cytoplasm of these senescent cells contained many stress fibers. Fibroblastic cells were observed in more than 90 percent of the cultures by the second passage.

Cells seeded in hormonally defined medium plated with 11 percent of the efficiency of those in maintenance medium. Each of the seven dog kidneys gave rise to epithelioid cell populations in all of the seeded flasks ($N = 61$). In the first week of primary culture, three morphologically distinct cell types were observed, all epithelioid. The first two types consisted of very large cells; one type had a clear cytoplasm (cell surface, 5268 ± 372 μm^2) (Fig. 1a), and the other had a granular and sometimes vacuolated cytoplasm (cell surface, 1659 ± 56 μm^2) (Fig. 1b). The third type consisted of small cells (cell surface, 588 ± 14 μm^2) (Fig. 1c) and was the only one that appeared to form domes (Fig. 1d). The ratio of these different cell types in primary culture was 1:3:4, respectively. Primary cultures were confluent within 2 weeks, but subsequent passages took 3 to 12 weeks to reach confluency. Split ratios larger than 1:2 or 1:3, when cells were approximately 80 percent confluent, produced cells that did not continue through the cell cycle. The maximum number of passages was three for any cell line, and no fibroblastic cells were observed in any of the cultures in hormonally defined medium. When isolated cells from the hormonally defined medium were switched to the maintenance medium (containing serum), growth was inhibited, but dome formation continued.

To determine whether dome formation was related to active sodium transport, we added ouabain at $10^{-5}M$ to each of three flasks that contained a number of domes. After 16 hours of exposure, no domes could be observed. After the flasks were thoroughly washed to remove ouabain, dome formation appeared to be similar in size and frequency to that observed before treatment.

Since the first demonstration that the serum component of a culture medium could be replaced totally by hormones (*16*), the hormonal requirements for many continuous lines have been defined (*17*). Because each of these continuous lines has its own requirements for optimal growth, it has been proposed that a hormonally defined medium could eventually be used to isolate and maintain growth of a specific cell type in primary culture (*3, 18*). Primary cultures of testicular and pituitary cells have been isolated from mice by use of media hormonally defined for continuous lines of the respective cell types (*19*); these isolated testicular and pituitary cells remain in culture for 3 to 4 weeks. Taub and Sato (*20*) initiated primary cultures of kidney epithelioid cells from other species. We were able to initiate and maintain canine kidney epithelioid cells in hormonally defined medium without any fibroblast contamination. Cells can be maintained in primary culture for up to three passages. Our cultures that have stopped growing at 6 months continue to form domes. Moreover, these canine cells have a transport function that can be inhibited by ouabain throughout the life of the cultures.

There is evidence (*21*) that serum inhibits the growth of both the collecting tubules and the thick ascending loop in primary cultures of rabbit kidney. These data, together with our own observations that growth is inhibited by the addition of serum-containing maintenance medium to cultures in hormonally defined medium, suggest that we have isolated an epithelium of either distal-tubule or collecting-duct origin. In fact, the MDCK line for which the hormonally defined medium was defined has properties resembling those of distal tubules or collecting ducts (or both) (*22*).

Transformed cells have reduced hormonal needs when compared to those that have not undergone transformation

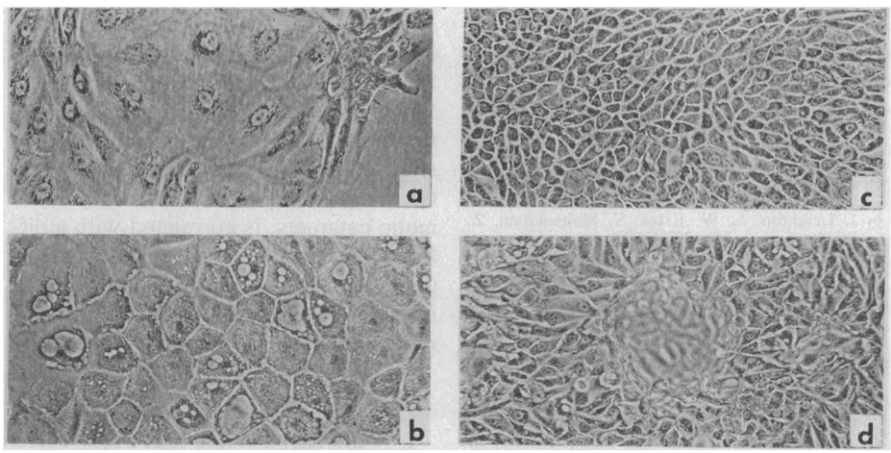

Fig. 1. After the removal of a small piece of renal cortex the tissue was washed in three changes of phosphate-buffered saline containing penicillin (250 unit/ml) and streptomycin (250 μg/ml). The tissue was minced in a few drops of dissociation medium, combined with 10 ml of dissociation medium, and incubated at 37°C for 1 hour, with agitation every 15 minutes. The dissociation medium was a mixture (1:1) of Ham's F-12 and Dulbecco's modified Eagle's medium containing CLS collagenase (0.5 mg/ml) (Worthington) and sterilized by filtration through a 0.45 μm filter. The resulting cell suspension was centrifuged at 240g for 10 minutes, and the pellet was washed twice with culture medium. Half of the pellet was placed in maintenance medium and the other half was placed in hormonally defined medium. Cells were grown in a closed system containing an atmosphere of 90 percent air and 10 percent CO_2. The cultures were kept at 37°C, and the medium was changed twice a week. Cells were passed by using a mixture of trypsin (5 mg/ml) and EDTA (2 mg/ml) in Hanks basic salt solution, Ca^{2+}- and Mg^{2+}-free. The tryptic activity in the cell suspension in hormonally defined medium was inactivated by a twofold excess of (by weight) soybean trypsin inhibitor. Cells were centrifuged at 240g for 10 minutes and were suspended in the appropriate medium. Phase contrast photographs of the cells (magnification ×125). (a) Large epithelioid cells with agranular cytoplasm. These cells almost totally disappear from culture following the first passage. (b) Large granular epithelioid cells. (c) Major cell type in primary cultures. Both (b) and (c) cell types are seen in a ratio of 1:1 following the first passage. (d) A dome structure. These domes are only found in areas similar to that shown in (c).

(*23*). Since the hormonally defined medium we used was designed for a continuous cell line, it is quite possible that our isolated "normal" cells may require additional hormones or factors (or both) to achieve a maximal growth rate. In summary, we have demonstrated that it is now possible to isolate and maintain a single kidney cell type that exhibits transport properties in a totally defined system.

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Persistence of Crystallin Messenger RNA's with Reduced Translation in Hereditary Cataracts in Mice

Abstract. *In vitro translation experiments showed that the lens fiber cells of two hereditary cataracts in mice (Nakano and Philly) possessed a full complement of crystallin messenger RNA's, despite severely reduced synthesis of crystallin in these cells. The reduction in synthesis in the lens fiber cells correlated with the increase in Na^+ and the decrease in K^+, which occurs during cataractogenesis. In contrast to the fiber cells, the epithelial cells continued to synthesize crystallins in the cataractous lenses. Crystallin synthesis was stimulated in the fiber cells by raising the K^+ concentration and lowering the Na^+ concentration in the cultured lenses. The reduction in crystallin synthesis in the initial stages of cataractogenesis in the Nakano and Philly lenses thus appears to be due to poor utilization of crystallin messenger RNA's in the fiber cells.*

Cataracts are lens opacities that seriously affect vision and may lead to blindness. One important initiating factor of cataractogenesis appears to be lens hydration, which is associated with a large increase in the intralenticular concentration of Na^+ and a decrease in the concentration of K^+ (*1*). Examples of osmotic cataracts with an elevated Na^+/K^+ ratio include galactose cataracts in rats (*2*) and hereditary cataracts in Nakano (*3*) and Philly (*4*) mice. Experiments with cultured embryonic chicken lenses have shown that an increase in the Na^+/K^+ ratio, comparable to that occurring in osmotic cataracts, is correlated with a differential reduction in crystallin synthesis (*5*), suggesting that crystallin synthesis may also be affected in mammalian cataracts associated with cation imbalances (*6*). This possibility was supported by experiments correlating a differential reduction in crystallin synthesis with alterations in the intralenticular concentration of Na^+ and K^+ in ouabain-treated normal (*7*), Nakano (*7*), and Philly (*8*) mouse lenses as well as in galactosemic (*9*) and hypocholesteremic (*10*) rat lenses. Differential reductions in protein synthesis have also been observed in human lenses cultured in the presence of ouabain (*11*). We now report that changes in Na^+ and K^+ affect crystallin synthesis by interfering with the efficient utilization of crystallin messenger RNA's (mRNA's) in mammalian cataracts.

Protein synthesis was examined in cultured lenses from 30-day-old normal (NIH) and Philly mice and from 40-day-old Nakano mice. By 30 days after

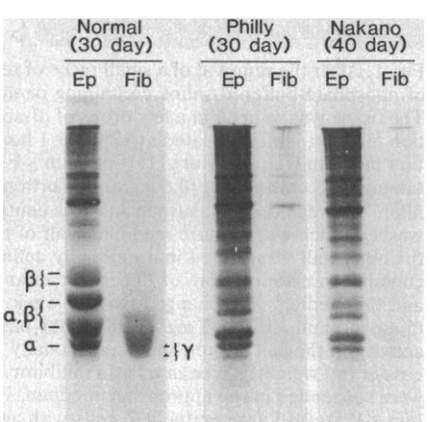

Fig. 1. Autoradiograms of sodium dodecyl sulfate (SDS)-urea-polyacrylamide gels of lens proteins from normal, Philly, and Nakano lenses. Three lenses were removed from the eyes and incubated in 0.5 ml of Ham's F-10 medium (*12*) supplemented with fructose (8.48 g/liter) and [^{35}S]methionine (New England Nuclear; 1 mCi/ml; 800 Ci/mmole) for 8 hours at 37°C in a humidified atmosphere of 5 percent CO_2 and 95 percent air. The epithelial cells were separated from the fiber cells under the dissecting microscope. They were dissolved in 0.75 ml of sample buffer (2 percent SDS, 8*M* urea, 1 percent 2-mercaptoethanol, 10 percent glycerol, 0.1*M* tris, *p*H 6.8) and the fiber cells were dissolved in 1.5 ml of the same buffer; 15 μl of the fiber cell homogenate and 30 μl of epithelial cell homogenate were subjected to electrophoresis in a 12 percent polyacrylamide gel slab and to autoradiography (*13*). The designation of the bands as α-, β-, or γ-crystallin was established by immunoprecipitation (*8*). The broad upper band, identified as γ-crystallin in the fiber cells, also contained α- and β-crystallin polypeptides.

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