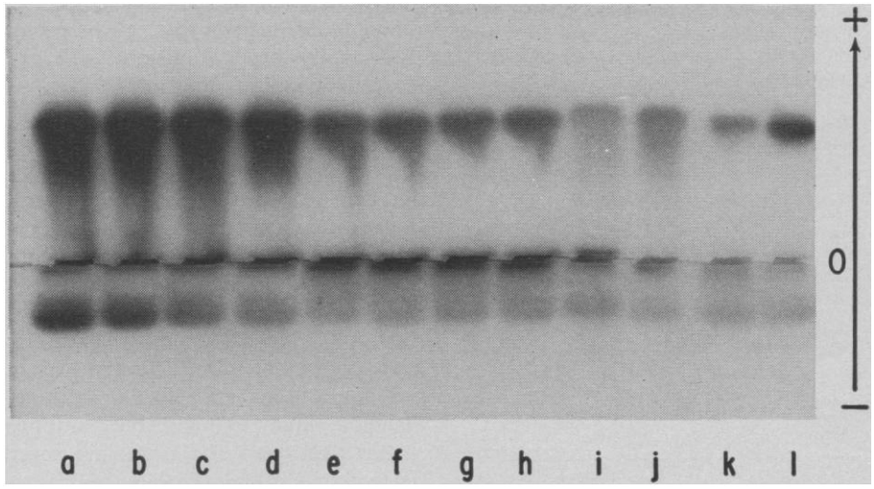

Fig. 3. Starch gel electrophoretic patterns of malate dehydrogenase isozymes (*O*, origin). Samples are (*a*) kidney tissue of control mouse, (*b, c, d*) kidney tissues of mice with neuroblastoma, (*e*) brain tissue of control mouse, (*f, g, h*) brain tissues of mice with neuroblastomas, (*i*) tumor induced by TH and ChA negative clone, (*j*) tumor induced by TH and ChA positive clone, (*k*) tumor induced by ChA positive clone, (*l*) tumor induced by TH positive clone.

tional slowest-moving band of esterase in all the tumor tissues (Fig. 2). The malate dehydrogenase pattern is not altered in any of the tissues observed (Fig. 3). Tumor, brain, kidney and muscle tissues all have the same type of distribution of malate dehydrogenase. All four clones of neuroblastoma cells have the same pattern of α-esterase and malate dehydrogenase.

The muscle type isozyme of lactate dehydrogenase (LDH-5) is the major form found in tissues exhibiting a high rate of glycolysis and is not inhibited by pyruvate (*7*). Thus the presence of only LDH-5 in neuroblastoma tumor cells may suggest that these neoplastic cells require as high a rate of glycolysis in vivo as they do in vitro. As there seems to be a significant correlation between the growth rates of some tumors (like hepatomas) and their glycolytic activity (*1*), the distribution pattern of LDH isozymes could also be significant in characterizing the growth of other types of tumors.

The significance of the presence of the extra esterase band in the tumors is not apparent. It could be speculated, however, that the extra activity of esterase is related to the anaerobic glycolysis in the sense that it helps liberate more glycerol from the fats. Glycerol is then utilized for the synthesis of either fructose or glucose, which in turn undergoes glycolysis.

Usually the B subunit (LDH-1) is high in brain and heart tissue (*8*). As neuroblastoma cells are also of neural origin one would expect the presence of a tissue-specific enzyme. The absence of B units and the presence of A units in the tumor cells suggest that expression of murine neuroblastoma is associated with a loss of specific neural enzymes and a replacement by other types which are normally either low or absent in the brain. Such phenomena of simultaneous "induction-repression" have also been observed in some hepatoma tumors (*9*). It has been shown that in early rat embryogenesis B polypeptide subunits of LDH are usually absent (*10*). Thus the absence of B units in neuroblastoma tumors and its presence in brain tissues suggest that certain embryonic properties reexpress in the neoplastic cells. This is consistent with the general observation that the malignant cells reexpress many embryonic features.

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Inherited Renal Cysts in Rats

Abstract. *A strain of rats that form renal cysts has been developed. The number of visible cysts increases with age after animals are 20 days old. Micropuncture studies indicate that the cystic fluid has a variable sodium concentration, but that the ratios of inulin concentration in tubular fluid to that in plasma are high.*

The maturation of renal function in neonatal rats has been studied by micropuncture technique in this laboratory (*1, 2*). Pregnant mothers were obtained from a commercial source (Simonsen, Gilvary, California), and micropuncture experiments were done on pups 15 to 55 days after birth. During these experiments, it was noticed that certain litters had kidneys with cysts visible on the kidney surface. Three sister-brother pairs were mated to determine if the cyst-bearing animals could be developed into a strain carrying cystic disease which could be an animal model for human polycystic disease (*3, 4*). Initial data reported here indicate that it has been possible to develop such a strain, and that further study could make contributions to our knowledge of cyst formation.

In the matings of two sets of parents (two litters each from pairs A and C),

Table 1. Number of surface cysts visible as rats develop.

Age (days)	Weight (g)	Sex	Cysts, left kidney (No.)	Cysts, right kidney (No.)
		Litter A_1		
22	44	♀	3	1
29	50	♂	3	3
		Litter B_1		
19	34	♂	0	0
19	30	♂	0	0
38	118	♂	2	1
45	118	♀	5–6	5–6
52	153	♀	0	0
		Litter C_1		
15	26	♂	0	0
27	55	♂	> 10	> 10
34	87	♀	3	4
41	136	♀	3	3
49*	168	♀	5	4
		Litter A_2		
17	29	♀	0	0
24	34	♀	0	0
25	50	♀	4	6
31	60	♂	4	4
39*	115	♀	4	5
45*	146	♀	6	3
		Litter C_2		
13	15	♂	0	0
21	32	♀	6	3
27	76	♀	8	4

* Kidney surface pitted in situ.

superficial renal cysts developed in all offspring examined after the age of 25 days (Table 1). In animals between 20 and 24 days old, two of three had cysts. In no case did any animals show cysts when they were less than 20 days old. Of five animals examined from a litter obtained from the other mating (pair B), only two were found to have visible cysts. These data are interpreted to indicate that the formation of cysts is genetically determined. The question as to whether the cystic trait is dominant, as has been suggested for the human disease (*3*), is not answered by these limited data. There does not seem to be a sex-related inheritance, since both sexes develop cysts.

Beyond the observation that cysts do not develop before animals are 20 days old, the time course of the renal morphological changes has not been investigated. It was noticed, however, that in three animals, 39, 45, and 49 days old, the kidney surface seemed pitted in situ, which suggests that there was nephron loss (Table 1).

In six other animals, micropuncture and other renal function studies were done during mannitol loading at an infusion rate of 0.5 ml of 10 percent mannitol in Ringer solution per hour. The studies done were measurement of glomerular filtration rate (four animals), micropuncture of normal-appearing tubules and cysts (five animals), micropuncture of normal and dilated proximal tubules (one animal), and measurement of plasma and urine Na and K (four animals). With the exception of measurement of tubular fluid electrolytes, the procedures for micropuncture have been described (*1, 5*). Measurements of tubular fluid Na and K were made by integrated flame photometry (*6*).

Table 2. Characteristics of cyst and dilated tubular fluid. Abbreviations: TF/P_{In}, ratio of inulin concentration in tubular fluid to that in plasma; TF_{Na}, tubular fluid sodium concentration; P_{Na}, plasma sodium concentration; T, tubule; and C, cyst.

Designation	TF/P_{In}	TF_{Na} (meq/liter)	P_{Na} (meq/liter)
	Rat 1: 27 days, 47 g		
T_1	44.9		
T_2	44.3	132	151
T_3	33.9		
	Rat 2: 24 days, 55 g		
C_{01}	21.4	89	135
C_{02}		188	134
	Rat 3: 25 days, 65 g		
C_{11}	7.12	155	142
C_{12}		104	142

No differences were evident as compared to noncystic controls with respect to kidney filtration rate, urinary Na and K excretion, or plasma electrolyte concentration; therefore, these data are not shown. Micropuncture studies of normal-looking proximal tubules did not yield different results than did comparable control studies. In one animal with no visible cysts, many broadly dilated tubules were evident and resembled those seen in diphenylamine-treated animals that have not yet developed visible surface cysts (*7*). These tubules were found to be proximal after later dissection. However, ratios of inulin in tubular fluid to that in plasma were extraordinarily high (rat 1 in Table 2). The fact that these ratios are greater than 1 is interpreted as indicating continued filtration and reabsorption in the dilated tubules, a conclusion previously reached for diphenylamine-treated precystic tubules (*7*).

In three animals, four cysts were punctured and fluid was collected. In two other animals, an additional four cysts were punctured but no fluid could be aspirated; this also occurred with another cyst of rat 2 in Table 2. These studies suggest that there may be two populations of cysts that can be classified on the basis of whether one can or cannot aspirate fluid.

Table 2 shows the results of the micropuncture studies of the cysts from which fluid was collected. Tubular fluid to plasma inulin ratios are all high as compared to ratios for control proximal tubular fluid (< 3.5) or for distal fluid samples taken under the conditions of these experiments (< 6.0) (*2*). Absolute sodium concentrations, as well as tubular fluid to plasma sodium ratios, are highly variable. Tubular fluid sodium concentrations higher than those of plasma have never been found in fluid from either proximal or distal tubules of mannitol-loaded rats. These data again show that when fluid can be aspirated from a cyst, the fluid is formed as a result of continued filtration and reabsorption. Whether reabsorption takes place across the walls of the cyst or at tubular sites in the nephron or nephrons leading to the cyst will be determined by future research.

The present studies establish that the disease is genetically linked but do not establish whether the disease in these animals is a reasonable model of human polycystic disease. However, the observations that the cysts develop only in animals over 20 days old and that the animals survive to reproduce would indicate that the cysts formed in this animal model are somewhat like the "mature" form of polycystic disease (*3*). Even if this model does not exactly parallel any human disease, the discovery of this strain should nevertheless prove of value in studies of the mechanism underlying cyst formation.

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