Renin Production by Organ Cultures of Renal Cortex

Abstract. *Diploid cells of the human and canine renal cortex can be isolated by newly developed techniques for organ culture. Some of these cells have abundant Bowie's positive granules. Bioassays for renin activity have shown that these granular cells can synthesize considerable amounts of renin in vitro. These investigations provide a model for studying the production of renin at cellular level.*

Experimental evidence suggests that the renal enzyme renin is associated with the glomerulus in the kidney (*1*) and that the stimuli that release renin also increase the granulation of juxtaglomerular cells (*2*). Immunofluorescent methods have demonstrated the high specificity of antirenin for these cytoplasmic granules (*3*). However, there are many questions concerning the mechanisms of synthesis and the release of renin at cell level, as well as the transport of the enzyme to the blood stream, that remain unanswered.

Newly developed methods of organ culture (*4*) have been used to investigate the production of renin by cells of isolated kidney cortex. We now report the successful growth of diploid cells of renal cortex in tissue culture under conditions in which they synthesize and release renin.

Table 1. Concentrations of renin found in pooled culture-medium supernatant in which kidney cells had grown and cultured kidney cells; each renin value is the average of at least three bioassays for each sample. Fresh cell medium had been applied to the growing cells every 120 to 400 hours. KS, kidney supernatant; KCH, kidney-cell homogenate; C, control.

Donor	Sample, type	Age of culture (hr)	Renin (ng/ml)
	Canine		
	KS + KCH		3.8
	KS		3.0
	C, medium		0.6
	Human		
D.W.	KS	715	15.4
J.M.	KS, left	186	25.8
J.M.	KS, left	454	4.2
J.M.	KS, left	507	1.3
J.M.	KS, left	843	1.3
J.M.	KS, left	1275	0.3
J.M.	KS, right	186	55.5
J.M.	KS, right	454	7.5
J.M.	KS, right	507	2.3
J.M.	KS, right	843	1.5
J.M.	KS, right	1275	0.7
J.M.	KCH, right	454	18.7, 20.2*
J.M.	KCH, left	454	30.4, 22.0*
K.C.	KS	95	23.0
K.C.	KS	167	6.0
W.M.	KS	699	0.9
W.M.	KS	774	0.9
	C, supernatant		0.6, 0.7, 0.3, 0.8*

* Duplicate samples.

Cells from human and canine renal cortex were obtained by open biopsy or by nephrectomy under strict sterile techniques. Blocks of tissue, approximately 3 by 2 by 2 mm, were rinsed in isotonic sodium chloride solution to eliminate blood and damaged cells and were transferred to glass-envelope-type cultures (*4*). The cultures were kept in Leighton tubes or 2-oz (60-ml) prescription bottles containing medium No. 199 (*5*) with 10 percent of autologous serum, 50 units of penicillin G, and 50 mg of dihydrostreptomycin per milliliter. The *p*H was adjusted to 7.2 and the cells grown at 37.2°C under an atmosphere of 5 percent of CO_2 in air or oxygen. The culture medium was changed every 5 to 15 days and cell growth and *p*H were observed daily. Supernatants from each group of cell cultures were periodically pooled and renin activities of the pools were determined in the following manner.

Cell suspensions from representative cultures were rinsed with saline, counted in a hemocytometer, broken by high-frequency sound at 4°C, and assayed for renin content. The number of granulated cells was determined after staining by Bowie's method (*6*) (Fig. 1). May-Grünwald-Giemsa stain was used for morphological studies. Karyotypes were determined by a modification of the method of Moorehead *et al.* (*7*).

Renin production was measured by means of a method similar to that used to measure renin activity in plasma (*8*). The tissue-culture medium or ruptured-cell suspension was dialyzed against ethylenediaminetetraacetic acid (2.2 g/liter) for 24 hours and then against distilled water for 24 hours. After dialysis, 5 ml of previously dialyzed human or dog plasma and one drop of diisopropyl fluorophosphate (1 : 20 in isopropanol) were added, the *p*H was adjusted to 5.5, and the mixture was incubated for 4 hours at 37°C. The *p*H was then adjusted to 5.0, and the solution was heated for 10 minutes in a bath of boiling water. The precipitated protein was removed by centrifugation, the *p*H was adjusted to 7.2 with sodium hydroxide and then to 6.8 with acetic acid, and the solution was dried in partial vacuum. The residue was then dissolved in saline, and the amount of angiotensin formed was measured by pressor assay in vagotomized rats treated with pentolinium. The results are expressed in nanograms of angiotensin formed per milliliter of original tissue-culture medium or cell suspension (Fig. 2). The pressor substance measured is most probably angiotensin; it was not present before incubation, it is destroyed by trypsin and chymotrypsin, and it has the same mi-

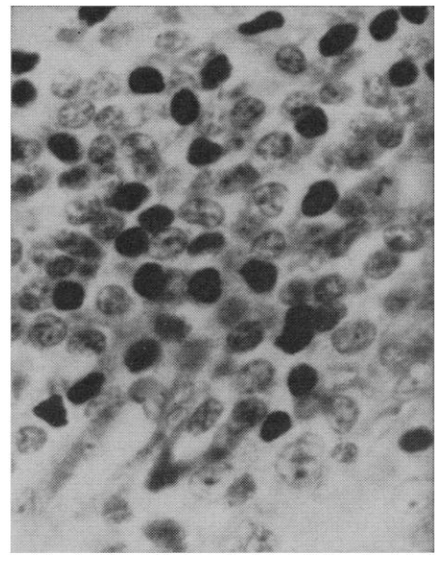

Fig. 1. Organ culture of cortex of human kidney after 454 hours. Granulated cells abound in a mixed population of epithelial, connective, and endothelial elements. Bowie's stain. (× 400)

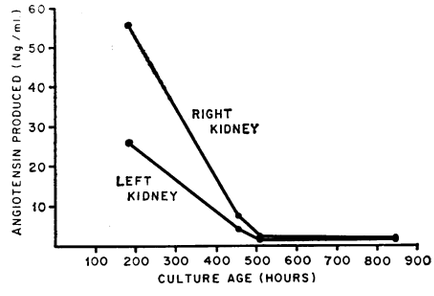

Fig. 2. Production of renin by left and right kidneys of the same human donor, expressed as nanograms of angiotensin produced by 1 ml of cell supernatant incubated for 4 hours with 1 ml of control plasma. Culture mediums were changed at 114 hours; bioassays were made at each subsequent feeding.

gration on paper chromatography as angiotensin in a mixture of *n*-butanol, acetic acid, and water (4:1:5).

Table 1 shows that such cells from canine and human kidney cortex produce and release renin into the tissue-culture medium. In general, renin concentrations are lower in supernatants from older cultures (see Table 1); the concomitant increase in number and size of Bowie's positive cytoplasmic granules in some of the cells suggests active intracellular synthesis of protein. Highest concentrations of renin were found in cultures of kidney cortex prepared from a patient with malignant hypertension (J.M.). In this experiment, in which cells from both kidneys were cultured, arteriolar nephrosclerosis was more severe in the left kidney although the two populations of cells were similar (Table 1). The cortical cells of the right kidney released more renin into the medium than cells of the left kidney. Only one culture came from a normal individual (W.M.) and it did not produce renin.

Cell morphology changed with time, cultures increasing significantly in the number of granulated cells after 600 hours. Morphological differences between epithelial cells from convoluted tubules and those with Bowie's positive granules were maintained in long-term cultures. Usually, cultures of human renal cortex grow faster than cultures of canine kidney.

Cultures of kidney obtained by biopsy from patients with various nephropathies are under study.

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Agouti Locus: Homology of Its Method of Operation in Rats and Mice

Abstract. *The action of genes at the agouti locus in rats is similar to that of genes at the same locus in mice—the color phenotype of hairs is determined by the operation of agouti-locus alleles in the follicle, rather than in the melanocytes. Hairs produced by mouse skin growing on partially tolerant infant rat hosts show that rat melanocytes also respond to the agouti-locus genotype of mouse follicles. This constitutes the first example in vivo of the behavior of a cell of one species being completely determined by the genotype of an alien host species.*

Mammalian coat coloration has long intrigued geneticists because it is a direct expression of gene action and interaction. One of the most widespread and interesting determinants of coat-color patterns is the *A* or *agouti* locus. Depending upon the specific alleles present at this locus, a series of phenotypes is produced with various distributions of pheomelanin (yellow) and eumelanin (black or brown) hair pigment. The agouti pattern, after which the locus in named, is characterized by yellow subterminal banding of the otherwise black or brown hairs over most of the body.

Although extensive studies on the action of genes at this locus have been carried out on mice (*1*), little information is available on the expression of agouti alleles in other species. Here we report that the action of genes at the *agouti* locus in the rat is the same as in the mouse.

To determine the primary site of action of the genes at the *A* locus in rats we studied the behavior of melanocytes of one agouti-locus genotype artificially introduced into developing hair follicles of a different agouti-locus genotype. Melanocyte substitution was achieved by transplanting skin from late fetuses to newborn animals under circumstances in which donor and host differed with respect to the nature (governed by agouti-locus genotype) and intensity (governed by other loci) of their future hair pigmentation. By this means advantage was taken of the fact that with animals of this age, host melanocytes migrate across the graft boundary and become incorporated in some of the developing follicles of the alien genotype. The pigmentation of emergent hairs from affected follicles indicates whether the genotype of the immigrant host melanocytes or that of the donor follicle apparatus determines the kind of melanin synthesized. An almost essential prerequisite for this approach is the absence, or feeble intensity, of pigment in the hairs of the graft donor's skin. This ensures that the melanocytes responsible for any intensely pigmented hairs which arise in the transplant must have been provided by the host.

Rats of the BN, DA, and Lewis strains and their hybrids were used. The mice originated from domestic sublines of strains A and BALB/c.

Rats of the BN strain are brown, non-agouti ($a/a;b/b$); DA rats, gray-bellied agouti (A^w/A^w); and those of the Lewis strain are albino, non-agouti ($a/a;c^a/c^a$). Lewis animals also carry genes for hooded (h/h) as well as silvering (s/s). This latter condition is also expressed to some extent in BN animals.

Lewis × DA F_1 hybrids served as agouti ($A^w/a;C/c^a$) hosts for histocompatible Lewis skin and for heterologous strain A ($a/a;c^a/c^a$) mouse skin. Lewis × BN non-agouti ($a/a;C/c^a$) animals received grafts from genetically compatible Lewis fetuses and from albino *but* agouti ($A^w/A^w;c^a/c^a$) donors—a stock derived from F_2 Lewis × DA albinos which were confirmed as being homozygous for A^w by test mating with BN animals. These non-agouti hybrid rats also served as hosts for BALB/c ($A/A;c^a/c^a$) mouse heterografts. The various donor-recipient combinations employed, together with their genotypes and phenotypes, are summarized in Table 1.

To make the infant Lewis × BN F_1 rats accept otherwise immunologically incompatible albino but agouti (A^w/A^w; c^a/c^a) skin homografts it was necessary to inoculate them intravenously at birth with 25×10^6 DA bone-marrow cells to render them immunologically tolerant of the foreign DA isoantigens possessed by these grafts (*2*). To weaken the infant rat hosts' resistance to mouse skin heterografts, and thus prolong the lives of the grafts, the hosts were irradiated with 300 r before being injected with 20 to 30 million mouse bone marrow cells from the prospective donor strain. They were grafted with mouse skin about 3 hours later.

Skin grafts were made from fetuses judged to be anywhere from 18 to 20