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.

ABEL L. ROBERTSON, JR.
ROBERT R. SMEBY
F. MERLIN BUMPUS, IRVINE H. PAGE
Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106

References and Notes

1. J. Bing and J. Kazimierczak, *Acta Path. Microbiol Scand.* **54**, 80 (1962); W. F. Cook and G. W. Pickering, *J. Physiol. London* **143**, 78P (1958).
2. L. Tobian, *Circulation* **25**, 189 (1962); *Can. Med. Assoc. J.* **90**, 160 (1964); J. A. Pitcock, P. M. Hartroft, L. N. Newark, *Proc. Soc. Exp. Biol. Med.* **100**, 868 (1959).
3. P. M. Hartroft, L. E. Sutherland, W. S. Hartroft, *Can. Med. Assoc. J.* **90**, 163 (1964).
4. A. L. Robertson and P. Puce, in preparation.
5. J. F. Morgan, H. J. Morton, R. C. Parker, *Proc. Soc. Exp. Biol. Med.* **73**, 1, (1950).
6. P. M. Hartroft and W. S. Hartroft, *J. Exp. Med.* **97**, 415 (1953).
7. P. S. Moorehead, P. C. Nowell, W. J. Hellman, D. M. Battips, D. A. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
8. P. T. Pickens, F. M. Bumpus, A. M. Lloyd, R. R. Smeby, I. H. Page, *Circulation Res.*, in press.
9. Supported in part by grants from the National Heart Institute (H-6835) and the American Heart Association. We thank Mrs. V. Bikerman and Paul Puce for technical assistance.

3 June 1965

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

Table 1. Phenotypes and genotypes of donor and host animals.

Donors (all phenotypes white)		Hosts		
Strains	Genotype	Strains	Genotype	Phenotype
Lewis	$a/a;c^a/c^a$	Lewis × DA	$A^w/a;C/c^a$	Gray-bellied agouti
Lewis	$a/a;c^a/c^a$	Lewis × BN	$a/a;C/c^a$	Black
(Lewis × DA)F_2	$A^w/A^w;c^a/c^a$	Lewis × BN	$a/a;C/c^a$	Black
A (mouse)	$a/a;c^a/c^a$	Lewis × DA	$A^w/a;C/c^a$	Gray-bellied agouti
BALB/c (mouse)	$A/A;c^a/c^a$	Lewis × BN	$a/a;C/c^a$	Black

days old, to recipients less than 12 hours of age after birth (*3*). All grafts, which were about 0.7 to 0.8 cm in diameter, originated from dorsal regions and were placed in the mid-dorsal region of the host. The grafts were examined daily under a dissecting microscope. In all instances pigmented graft hairs, as well as host hairs, were plucked and examined microscopically (*4*).

In the intraspecific rat combinations tested, graft hairs began to emerge on the 12th day after surgery. Usually they were devoid of pigment. However, within a few days many pigmented hairs appeared within the graft. Most of them were located immediately within the graft border, but some were found well within its perimeter, intermingled with the characteristic nonpigmented donor hairs. These secondarily pigmented hairs increased in number so rapidly that by 25 days the pigmentation of the fur on the graft was sometimes discernible to the naked eye. Indeed, some grafts ultimately acquired as many pigmented as nonpigmented hairs.

In all instances the color of graft hairs populated by host melanocytes was determined by the agouti-locus genotype of the follicles. Thus, in six Lewis grafts to Lewis × BN (non-agouti) recipients, black hairs, or black-white mosaic hairs, emerged among the indigenous, nonpigmented hairs within the graft border. A similar situation occurred in eight Lewis grafts to Lewis × DA (agouti) animals. Eight Lewis × BN (non-agouti) hybrids tolerant of albino but agouti (A^w/A^w; c^a/c^a) grafts displayed agouti hairs among the white hairs. A number of completely black guard hairs were found as well, but these also occur normally in agouti animals.

As anticipated, and unlike rat skin homografts, mouse skin heterografts did not live very long on the rat hosts. Of 38 heterografts only 10 exhibited some evidence of epithelial survival at 20 days and in no instance did a mouse graft survive longer than 25 days on a rat. However, inasmuch as pigmented graft hairs were first observed as early as the 12th day after grafting, the short life-span of these heterografts did not preclude appraisal of the fate of rat melanocytes in mouse follicles.

The color of the pigmented hairs that originated within these heterografts was again determined by their agouti-locus genotype. Thus when strain A (albino but non-agouti) mouse skin was transplanted to Lewis × DA (agouti) rat hybrids, four animals displayed some black or gray hairs within the border of the graft. The greatest number of these hairs in any single recipient was about 10 to 12 and, in most instances, they were predominantly mosaic in nature, containing a reduced number of black granules. In all eight cases in which pigmented hairs were found within the border of albino but agouti mouse grafts (furnished by BALB/c animals) on non-agouti, Lewis × BN hosts, they were typical agouti in pattern, exhibiting black tips with yellow bands. Some of these hairs originated from grafts which were rejected while they were in the yellow phase but others survived long enough to revert back to eumelanin production. Many of these pigmented hairs were deficient in pigment granules, containing either a reduced number of granules in each hair septule, or, completely lacking granules over a portion of the shaft. This deficiency of pigment, like that noted in other combinations, was undoubtedly the result of the incorporation of only a few melanoblasts in the hair follicle. A few black hairs were also observed originating from these grafts but, as already stated, they are also found naturally in agouti animals. The number of graft hairs pigmented by host cells varied from two or three to more than 30.

These findings indicate that the primary action of genes at the agouti locus of the rat is the same as in the mouse—that is, in the hair bulb and not in the melanocyte. Furthermore, the agouti locus in these two species is apparently homologous inasmuch as rat melanocytes can respond to the agouti-locus genotype of mouse hair follicles. This situation is certainly unique and, as far as is known, represents the first example in vivo where the behavior of a cell of one species is completely determined by the genotype of an alien host species.

WILLYS K. SILVERS
Department of Medical Genetics,
University of Pennsylvania
School of Medicine, Philadelphia

References and Notes

1. W. K. Silvers and E. S. Russell, *J. Exptl. Zool.* **130**, 199 (19[illegible] W. K. Silvers, *ibid.* **137**, 181 (1958) —— *ibid.* **137**, 189 (1958); G. Cleffman, *Exptl. Cell Res.* **35**, 590 (1964).
2. R. E. Billingham, in *Transplantation of Tissues and Cells*, R. E. Billingham and W. K. Silvers, Eds. (Wistar Institute Press, Philadelphia, 1961), p. 87.
3. D. Steinmuller, *ibid.*, p. 27.
4. E. S. Russell, *Genetics* **31**, 327 (1946).
5. Supported in part by PHS grant CA 05927 and by a PHS career development award. The technical assistance of Mrs. Barbara Davis and George Sawchuck is gratefully acknowledged. I am also indebted to R. E. Billingham for helpful criticism and advice.

17 May 1965

Progesterone: Biosynthesis from Pregnenolone in Holarrhena floribunda

Abstract. *After administration of pregnenolone-4-C^{14} to* Holarrhena floribunda *leaves, radioactive progesterone was isolated and purified to constant specific activity by chromatography, conversion to a derivative, and recrystallization. The result suggests that the biogenetic sequence leading to progesterone is the same in plants as in animals.*

The discovery of the animal hormone progesterone in the African plant *Holarrhena floribunda* (*1*) has led us to study the biosynthesis of this compound. In animals, progesterone is formed directly from pregnenolone (*2*). The latter has thus far been found in only two plants, *Xysmalobium undulatum* (*3*) and *Trachycalymna fimbriatum* (*4*). However, pregnenolone-21-C^{14}-glucoside was converted to digitoxigenin and other cardenolides by *Digitalis lanata* leaves (*5*), and Tschesche and Snatzke (*3*) have suggested that pregnenolone may serve as a precursor of the steroidal estrogens found in plants (6), as it does in animals (*2*). We have now isolated