peritoneal macrophages; the supernatant fluids also inhibited the uptake of oxygen by porcine kidney cells. Virulent and avirulent *L. pomona* were equally toxic. The soluble toxic factor was nondialyzable and thermolabile; it was not specifically neutralized by antiserum.

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Genetic Regulation of Multiple Forms of Tyrosinase in Mice: Action of *a* and *b* Loci.* (32463)

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The relationship of tyrosinase activity to the qualitative and quantitative attributes of melanin granules (melanosomes) found in the various coat color mutants of mice has received careful attention(1-3). It is generally held that alleles at the *c*-locus regulate tyrosinase activity by direct involvement in the production of tyrosinase molecules(1,2). Alleles at the *b*-locus influence not only tyrosinase activity but also the nature of eumelanin (black *vs* brown) synthesized by melanocytes(1,2). It has been proposed that the *b*-locus controls, at least in part, the production of the protein matrix which forms an intricate component of the melanosome (1,2). The matrix appears to provide binding sites for the tyrosinase units synthesized in conformity with *c*-locus specifications. Alleles at the *a*-locus determine the type of melanin (phaeomelanin *vs* eumelanin) produced within the melanosome and also appear to have influence on the structure of its protein matrix (1,4).

As in a variety of other organisms, the tyrosinase of mammals has been shown to occur in a number of forms separable by electrophoresis(1,5-9). Considerable attention has been directed toward elucidating the properties of tyrosinase in melanomas derived from mice and hamsters(5-12). Recently, Wolfe and Coleman(1) reported the existence of multiple forms of tyrosinase in the normal skin of pigmented mice. In their study, involving allelic substitutions at the *c*-locus, a maximum of two electrophoretically separable forms of tyrosinase were identified. Alleles at the *c*-locus appeared to influence the presence or absence of specific bands of tyrosinase and also their mobility. This first demonstration of an association between genetic mechanisms and the varieties of tyrosinase obtained from normal melanocytes has added a new dimension to mammalian pigment cell research. The obvious need for more complete insight

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into the extent of this genic involvement has led to the present investigation. Extensive allelic substitutions at the *a* and *b* loci in the mouse reveal that the development of multiple forms of tyrosinase is subject to complex genetic control.

Methods. All mice were 8 weeks of age at the start. Both male and female mice were used since preliminary studies revealed no significant influence of sex on the results obtained. The strains of color stocks used included: (Except where necessary only mutant genes are listed) "wild-type" = *A/A, B/B, C/C*) C57BL/(sublines: 6J, St) (*a/a*), LT/Ch (*a/a,B*^{lt}*/B*^{lt}), C57Br/Jax (*a/a,b/b*), cordovan (*a/a,b*^{c}*/b*^{c}), beige (*a/a,bg/bg*), (C57BL × LT)F$_1$ (*a/a,B*^{lt}*/B*), (C57Br × LT)F$_1$ (*a/a,B*^{lt}*/b*), Y/Wi (*A*^{y}*/A*) and (*A/A*), L/St (*A*^{w}*/A*^{w}*,c*^{ch}*/c*^{ch}), (C57BL × C57Br)F$_1$ (*a/a,B/b*), and BUB/Wi (*a/a, c/c*). Hair growth was initiated by plucking quiescent hairs from the entire dorsum of each mouse; the animals were sacrificed by cervical dislocation approximately 10 days after plucking, a time when the hair tips were just emerging above the skin surface. The skin from the plucked region was removed and the panniculus carnosus dissected from the inner surface. The hair bulbs, thus exposed, were scraped off and frozen immediately on dry ice. Hair bulbs from 2 to 6 mice were pooled yielding preparations which ranged in weight from 0.4-1.5 g. Each sample was homogenized with a Potter-Elvehjem glass homogenizer in 4-5 ml of 0.25 M sucrose. The homogenate was centrifuged for 30 minutes at 34,500 × *g* and 0°C. Aliquots of the supernatant (0.2-0.3 ml) were then subjected to acrylamide-gel electrophoresis according to the method of Davis(13) with the exceptions that the upper and lower bath buffers were not diluted and the sample gel was omitted; the enzyme preparation was layered directly on top of the the spacer gel. The running time for the electrophoresis was 20-40 minutes at 3-5 ma/tube. The gels were neutralized with 1 M phosphate buffer, pH 6.7, for 30 minutes and the multiple forms of tyrosinase visualized by incubation in a solution of 0.1 M phosphate buffer, pH 6.8, containing 0.15% L-3,4-dihydroxyphenylalanine (L-DOPA) for several hours. To preserve the tyrosinase patterns, the gels were stored in 7½% acetic acid.

Results. As indicated in Table I, depending on the genotype of the mice, tyrosinase extracted from hair bulbs is found to exist in as many as 3 distinct molecular forms separable by acrylamide-gel electrophoresis. The location of the diverse tyrosinases in the acrylamide-gel is demonstrated by the deposition of dark melanin pigment at restricted sites on incubation in DOPA-reagent (Fig. 1 and 2); each tyrosinase band has a characteristic R_x value. The fastest moving band has been designated as T_1, with R_x values ranging from .661 to .697; the intermediate band as T_2 (R_x: .559 to .572); and the slowest as T_3 (R_x: .521 to .541). Allelic substitutions at the *b*-locus do not significantly alter the R_x values of any of the 3 bands. Judging from color intensity, all combinations of alleles at the *b*-locus, with the exception of brown (*b/b*), produce no significant differences in DOPA-reactivity among the three bands (Fig. 1 and 2A). In the brown (*b/b*) genotype, however, the slower moving tyrosinases, T_2 and T_3, are considerably less reactive with DOPA, for these bands are significantly lighter in color than T_1; in fact, T_2 is barely demonstrable (Fig. 1B).

With regard to the *a*-locus, in the presence of the yellow (*A*^{y}) allele, there is a marked reduction in tyrosinase activity within the

TABLE I. Effect of Genotype on R_x Values of Soluble Tyrosinases in Acrylamide-gel.

Locus a	Locus b	Locus c	R_x* T_1	R_x* T_2	R_x* T_3
a/a	B/B		.689	.569	.538
a/a	B/B^{lt}		.689	.568	.534
a/a	B/b		.689	.571	.537
a/a	B^{lt}/B^{lt}		.661	.560	.528
a/a	B^{lt}/b		.676	.572	.541
a/a	b^{c}/b^{c}		.697	.563	.563
a/a	b/b		.674	.559†	.524†
a/a	bg/bg		.689	.561	.521
A^{y}/A	B/B		.640	—	—
A/A	B/B		.690	.570	.530
A^{w}/A^{w}	B/B	c^{ch}/c^{ch}	.700	.590	.530
‡a/a	B/B	c/c	—	—	—

* Average values (5-6 gels/genotype).
† Bands were lighter compared to T_1.
‡ Albino control.

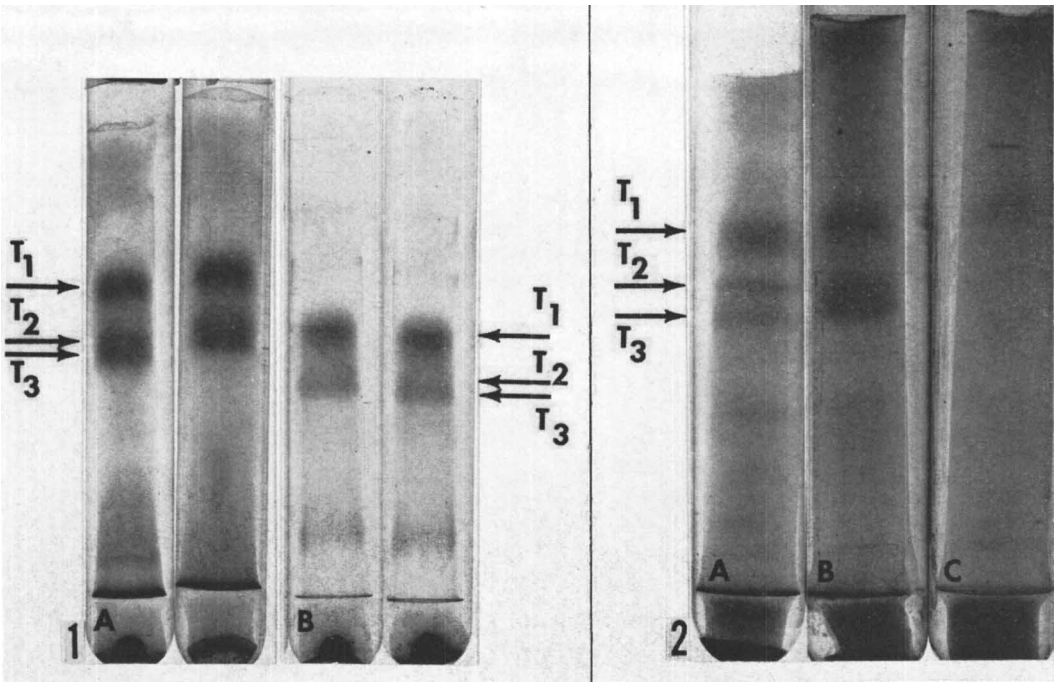

FIG. 1. Activity patterns of tyrosinase from follicular melanocytes; (A) Black (a/a, B/B); (B) Brown (a/a, b/b).

FIG. 2. Activity patterns of tyrosinase from follicular melanocytes; (A) Light (a/a, B^{lt}/B^{lt}); (B) Black Agouti (A/A, B/B) assayed when black melanin was being synthesized; (C) Yellow (A^y/A, B/B).

gels. A weak T_1 band is evident, but the T_2 and T_3 bands cannot be discerned (Fig. 2C). In contrast, agouti (A) hair bulb extracts show a normal tri-banded "black" tyrosinase pattern when the hair bulbs are obtained at the 10th day of the hair cycle, a time when black melanin is being formed by follicular melanocytes (Fig. 2b). The tri-banded "black" tyrosinase pattern is also characteristic of extracts prepared from hair bulbs removed from white-bellied, agouti-chinchilla (A^w/A^w, c^{ch}/c^{ch}) mice at the 10th day of the hair growth cycle. No evidence of tyrosinase activity is found in extracts from albino (c/c) hair bulbs.

Discussion. Alleles at the a and b loci in mice dramatically influence the occurrence and activity of multiple forms of tyrosinase separable by electrophoresis. As yet, it is not clear whether distinct species of tyrosinase molecules are involved or whether different "carrier-proteins" are associated with a common active enzyme component. In addition, it remains to be determined whether the various forms of tyrosinase are freely soluble or "particle bound" within melanocytes.

Based on the current knowledge of tyrosinase synthesis and the postulated action of the b-locus(2), one might speculate that the band T_1 represents unbound soluble tyrosinase destined for incorporation into melanosomes. The two slower moving bands, T_2 and T_3, may reflect tyrosinase which has been incorporated into melanosomes and linked to specific proteins that serve as structural components of the melanosome matrix. Thus, the "carrier-proteins" of T_2 and T_3 might represent structural elements of the melanosome which are liberated along with tyrosinase when tissue homogenates are prepared. The variant T_2 and T_3 profile of b/b extracts may reflect differences in the binding relationships of tyrosinase with the protein matrix programmed by alleles at the b-locus. Although this proposal is consistent with all available information, other hypotheses might be applied with equal validity.

The failure to demonstrate significant T_2

and T_3 tyrosinase activity in enzyme preparations from yellow mice cannot be readily explained. The biosynthetic pathway involved in the production of phaeomelanin (yellow pigment) is incompletely known, although tyrosine appears to be at least one of the precursors involved(14). It is possible that the absence of T_2 and T_3 reflects basic differences in the synthesis of phaeomelanin and eumelanin, or differing binding relationships between enzyme and protein matrix.

It is noteworthy that in both brown and yellow melanosomes structural defects in the protein matrix are a characteristic feature(2, 4). A relationship may exist between such alterations in structure and the variations in tyrosinase patterns reported here.

Summary. Extensive allelic substitutions at the *a* and *b* loci in mice reveal that multiple forms of tyrosinase separable by acrylamide-gel electrophoresis are subject to complex genetic control. Depending upon genic constitution at the *a* and *b* loci, a maximum of three electrophoretically separable forms of tyrosinase are demonstrable. Specific alleles at these loci have also been shown to influence tyrosinase activity.

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A Study of the Orifice of the Human Coronary Artery.* (32464)

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The origin of the coronary artery is a common site for atheroma formation and obstruction at the coronary orifice is one recognized cause of myocardial infarction(1). This is an area where two vessels with different architecture are joined and little is known of its physical properties, although extensive measurements of physical properties have been made *in vitro* and *in vivo* upon the aorta, and its major branches(2,3). A postmortem study of the coronary orifice has been performed to ascertain whether its physical properties resemble the aorta, one of its major branches, or some more peripheral vessel. The effect of calcium removal has also been studied for comparison with previous results obtained in the femoral artery.

Materials and methods. Aortas were obtained at autopsy from 10 men and 4 women aged 51 to 88 years. The aortic valve was removed intact and separated from the ascending aorta. Each coronary orifice and a surrounding cuff of aortic wall was dissected out. Tension-length diagrams were obtained with an apparatus used previously to study human femoral arteries(4). It was modified

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