INFLUENCE OF MATERNAL PHENOTYPE ON METABOLIC DIFFERENTIATION OF AGOUTI LOCUS MUTANTS IN THE MOUSE

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ABSTRACT

The results of extensive breeding experiments indicate that the phenotypic differentiation of embryos carrying the viable yellow, A^{vy}, or mottled, a^m, mutations is influenced to a major extent by the agouti locus genotype and the strain genome of the dam. The A^{vy}/a and a^m/a genotypes are each expressed in a spectrum of coat color phenotypes. These can be grouped into two classes, mottled and pseudoagouti.—In a reciprocal cross of C57BL/6JNIcrWf and AM/Wf-a^m/a^m mice, 29.5% of the offspring of C57BL/6 dams were of the pseudoagouti phenotype, whereas no pseudoagouti offspring were produced by AM strain dams.—Mottled yellow A^{vy}/a mice become obese and tumor formation is enhanced in these mice in comparison with the lean pseudoagouti A^{vy}/a siblings.—In two different reciprocal crosses using four different inbred strains, the proportion of pseudoagouti A^{vy}/a offspring differed according to the strain of the dam. Regardless of strain, mottled yellow A^{vy}/a dams produced significantly fewer pseudoagouti A^{vy}/a offspring than did black a/a dams.—The data suggest that metabolic differentiation of A^{vy}/a zygotes into phenotypic classes with different susceptibilities to obesity and tumor formation is influenced to a considerable degree by the metabolic characteristics of the oviductal and uterine environment of the dam.

DIFFERENTIATION of the metabolic milieu of local tissue environments is influenced to a major degree by the agouti locus on Chromosome *2* of the house mouse, *Mus musculus*. Mutations at this locus exert their effects on the coat color pattern by alteration of the hair-bulb environment of the melanocytes (SILVERS 1961). The hair-bulb environment regulates the periodic synthesis of eumelanin and/or phaeomelanin by the same melanocytes. The distribution of diverse hair-bulb environments over the body of the animal is determined by the agouti locus, as indicated by the distinct patterns of yellow, agouti (eumelanic hair with a subterminal phaeomelanic band), and black or brown hairs in different areas of the body of agouti-locus mutants (SEARLE 1968). The size of the hair bulb also influences the pigment pattern within specific hair types (GALBRAITH 1969).

Specific agouti-locus alleles can alter the type of pigment deposited in hairs growing from hair bulbs of a specified size (GALBRAITH and WOLFF 1974). In addition, regional differences in the dermis influence the type of pigment deposited in the hairs (POOLE and SILVERS 1976).

Two mutations, viable yellow (A^{vy}) and mottled (a^m), induce extreme lability in the metabolic differentiation of local tissue environments as expressed in a wide spectrum of coat color phenotypes.

The viable yellow heterozygous genotype, A^{vy}/a, is expressed in coat color phenotypes ranging from clear yellow, through all degrees of black and agouti mottling on a yellow background, to mice in which the coat-color pattern mimics, grossly, the wild-type agouti pattern. The latter are designated "pseudoagouti." Among the mottled phenotypes, no constant patterns are discernible; the mottling appears to occur randomly over the animal.

Mottled yellow A^{vy}/a mice start to become obese about puberty (Figures 1 and 2) and, as adults, are more susceptible to tumor formation than their nonyellow littermates. In contrast, the pseudoagouti A^{vy}/a phenotype does not become obese and susceptibility to spontaneous hepatoma formation is not greater than that of their nonyellow (a/a) littermates (WOLFF and PITOT 1972). Suspensions of Sarcoma 37 and Ehrlich-Lettre Hyperdiploid ascites cells implanted subcutaneously form smaller and lighter solid tumors in pseudoagouti A^{vy}/a mice than in mottled yellow A^{vy}/a littermates (WOLFF 1971).

Mottled heterozygotes, a^m/a, also exhibit a continuum of coat-color patterns ranging from all-black mice, indistinguishable from mice of the extreme nonagouti genotype (a^e/a^e), through various degrees of yellow mottling on a black background, to individuals with the agouti coat color pattern. These pseudoagouti a^m/a mice are indistinguishable from the pseudoagouti A^{vy}/a mice except by breeding tests.

The pseudoagouti and mottled yellow A^{vy}/a phenotypes differ in numerous physiological parameters (WOLFF 1971). These differences probably reflect the considerable divergence between the two phenotypic classes in the regulation of metabolic processes related to lipid metabolism and normal and neoplastic growth (WOLFF 1971). Generalized systemic metabolic differences between the pseudoagouti and mottled $a^m/—$ phenotypes, if they exist, are not obvious. The growth curves of both phenotypes are identical (Figure 2); however, no other parameters have been studied.

Previous data (WOLFF 1971) suggested that the frequency of appearance of pseudoagouti A^{vy}/a mice was, at least in part, dependent on the background genome. The present report demonstrates the strong influence of the maternal phenotype on the differentiation of A^{vy}/a and a^m/a zygotes. Some of these data have been presented previously (WOLFF 1976).

MATERIALS AND METHODS

Five inbred mouse strains were used in this study: AM/Wf, AT/Wf, C57BL/6JNIcrWf, VY/Wf, and YS/ChWf-A^{vy}. All strains, except C57BL/6, are maintained with forced heterozygosis at the agouti locus, *viz.*: AM/Wf-A^{vy}/a^m, a^m/a^m; AT/Wf-A^{vy}/a^t, a^t/a^t; VY/Wf-A^{vy}/a, a/a; YS/ChWf-A^{vy}/a, a/a. The origins of the VY and YS strains were described previously (WOLFF and PITOT 1973). In 1962 the A^{vy} gene was placed on the YS strain back-

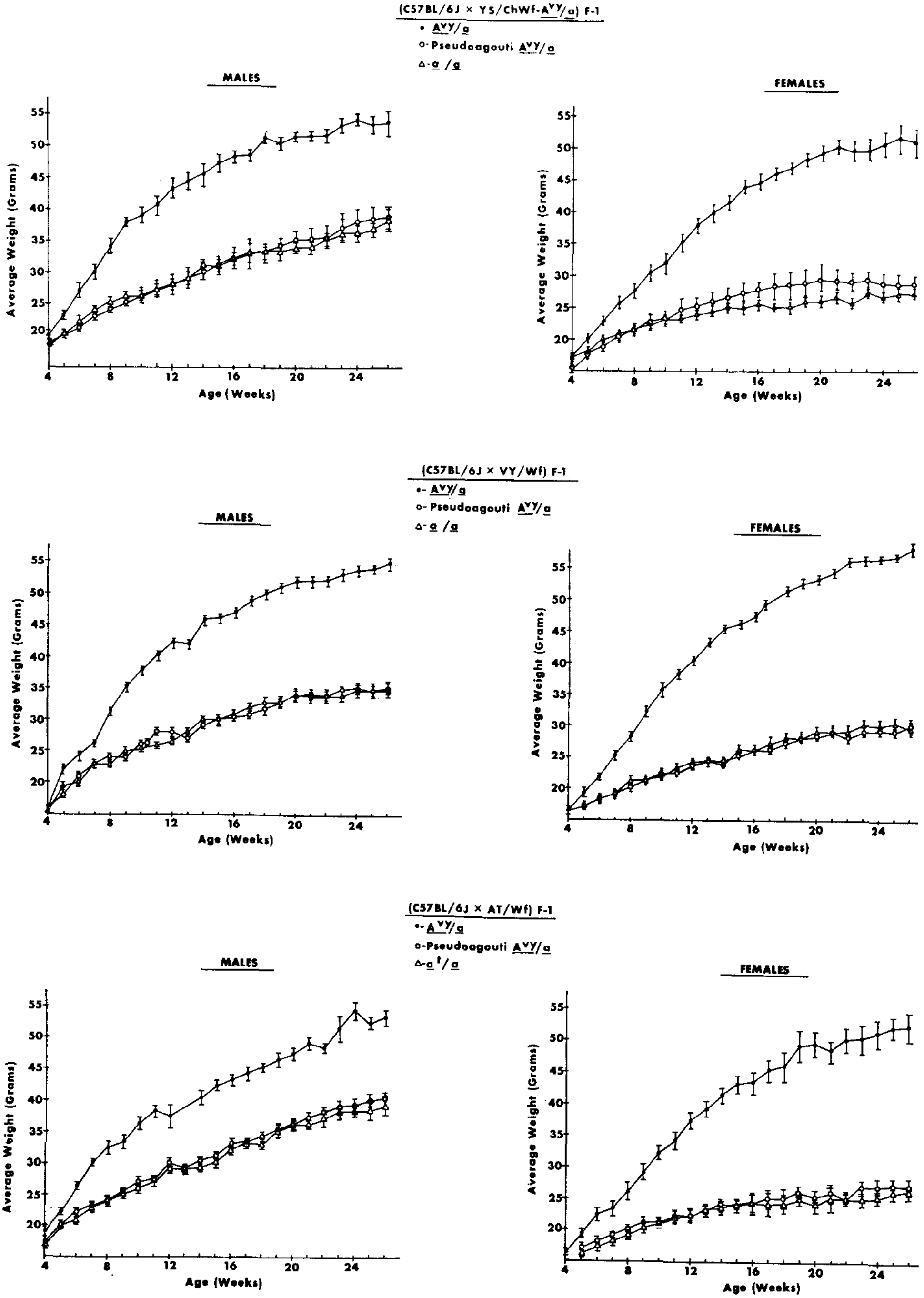

FIGURE 1.—Growth curves of mottled yellow, pseudoagouti (A^{vy}/—), and nonyellow male and female mice of three F_1 hybrids.

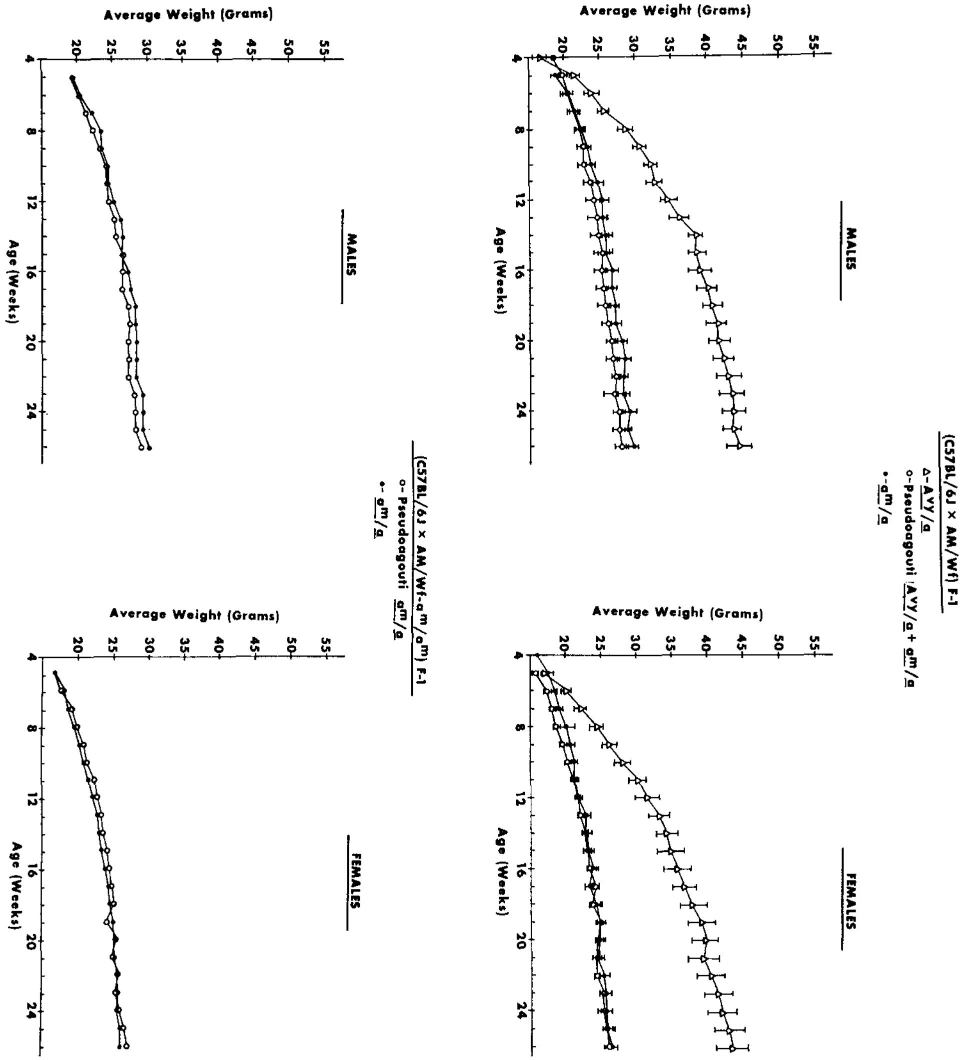

FIGURE 2.—Growth curves of mottled yellow A^{vy}/a^m, pseudoagouti A^{vy}/a^m and a^m/a, and mottled a^m/a male and female F_1 hybrid mice.

ground by mating a third backcross generation C57BL/6J-A^{vy} male to three YS/ChWf-a/a sisters. Subsequently, A^{vy}/a offspring have been continously backcrossed to YS-a/a mates.

The AM/Wf strain originated with an a^m/a male obtained in 1965 from DR. L. B. RUSSELL, Oak Ridge National Laboratory. This male was mated to an A^{vy}/a female of the VY strain in the F-12 generation; subsequently the strain has been maintained by matings of A^{vy}/a^m with a^m/a^m sibs.

The AT/Wf strain was started in 1966 by mating a black-and-tan (a^t/a) female of the YS/ChWf-a^t backcross stock, in the eighth generation of backcrossing, to a yellow (A^{vy}/a) male of the VY strain in the F-13 generation. It has been maintained since by sib mating A^{vy}/a^t by a^t/a^t mice.

The C57BL/6JNIcrWf substrain was derived from the F-36 generation of the C57BL/6JNIcr substrain.

Four mutations at the agouti locus were included: A^{vy} (viable yellow), a^t (black and tan), a^m (mottled), and a (nonagouti). The A^{vy} mutation arose in the C3H/Di substrain (DICKIE 1962) and the a^m mutation appeared among the offspring of a radiation experiment (RUSSELL 1964).

All inbred and F$_1$ hybrid stock mice, except AM/Wf, received Purina Laboratory Chow #5017 (6% fat) *ad libitum*. Stock mice of the AM/Wf strain received Purina Laboratory Chow #5010C (5½% fat). All pregnant and nursing females, except AM/Wf, were fed a 1:1 mixture of Purina #5017 (6% fat) and #5015 (11% fat); AM/Wf dams received a 1:1 mixture of Purina %5010C and #5017.

Hyperacidified (pH 2.5) water was always available. Room temperature was maintained between 21° and 27°, and relative humidity was about 45–55%. Microenvironmental temperature fluctuation was minimized by covering each 6½" × 11½" polycarbonate cage with a molded design filter bonnet of spun bonded polyester. Hardwood chips supplied by Absorb-Dri, Inc. were used as bedding.

The automatically controlled light cycle provided 12 hr of light.

RESULTS

Initially, the distribution of pseudoagouti A^{vy}/a offspring in litters born to yellow mothers mated by nonyellow males and those born to nonyellow dams mated by yellow sires was examined in three inbred strains—YS/ChWf, VY/Wf, AT/Wf. These litters were all born between January 1975 and April 1976. As is apparent from Table 1, yellow dams in the YS/ChWf and VY/Wf strains produced almost no pseudoagouti offspring, whereas black dams produced 11–12% pseudoagouti young among their A^{vy}/a progeny. Among AT/Wf strain matings, yellow females produced only about 5% pseudoagouti young among their A^{vy}/a offspring, whereas the A^{vy}/a offspring from black and tan (a^t/a^t) females included about 25% pseudoagouti young. All of these differences were significant at $P < 0.001$ when tested by the chi-square test for independence.

In order to ascertain whether the pseudoagouti phenotype had any effects on the differentiation of the A^{vy}/a zygotes, the complete breeding data on the YS/ChWf and VY/Wf strains since their transfer to the National Center for Toxicological Research in 1972 were analyzed according to type of mating. These data are presented in Table 2. In the VY/Wf strain, yellow A^{vy}/a females produced the lowest proportion of pseudoagouti offspring, pseudoagouti A^{vy}/a females

TABLE 1

Proportion of pseudoagouti mice among $A^{vy}/–$ offspring from yellow and nonyellow dams in three inbred strains

			$A^{vy}/–$ offspring			
	Genotypes		Pseudoagouti		Mottled yellow	
Strain	Dam	Sire	%	N	%	N
YS/ChWf	A^{vy}/a	a/a	0.9	2	99.1	219
	a/a	A^{vy}/a	10.8	23	89.2	191
VY/Wf	A^{vy}/a	a/a	0	0	100	185
	a/a	A^{vy}/a	12.6	32	87.4	221
AT/Wf	A^{vy}/a^t	a^t/a^t	4.9	15	95.1	291
	a^t/a^t	A^{vy}/a^t	24.6	84	75.4	258

TABLE 2

Production of pseudoagouti mice by dams of different phenotypes in two inbred strains

Strain	Genotypes Dam	Sire	A^{vy}/a offspring Pseudoagouti %	N	Mottled yellow %	N
YS/ChWf-A^{vy}	A^{vy}/a	a/a	0.3	2	99.7	605
	$^*A^{vy}/a$	a/a	1.2	4	98.8	325
	a/a	A^{vy}/a	16.8	49	83.2	243
	a/a	$^*A^{vy}/a$	16.9	72	83.1	355
VY/Wf	A^{vy}/a	a/a	1.0	17	99.0	1689
	$^*A^{vy}/a$	a/a	5.3	15	94.7	266
	a/a	A^{vy}/a	12.0	272	88.0	2024
	a/a	$^*A^{vy}/a$	10.1	60	89.9	533

* Pseudoagouti.

produced a somewhat higher proportion of pseudoagouti young ($P < 0.001$), and black *a/a* females produced the highest proportion of pseudoagouti offspring ($P < 0.001$) regardless of whether they were mated to yellow or pseudoagouti males.

The proportions of pseudoagouti young produced by yellow and pseudoagouti YS/ChWf dams were not significantly different from the proportions found in the VY/Wf strain. It is interesting to note, however, that the proportion of pseudoagouti young produced by the black YS females was significantly greater ($P < 0.001$) than the proportion produced by black VY dams. This difference between black females of the two strains was confirmed by the results of reciprocal crosses between these strains. Matings of VY–a/a females by YS–A^{vy}/a males produced about 10% pseudoagouti young among the A^{vy}/a offspring, whereas YS–a/a × VY–A^{vy}/a matings resulted in about 21% pseudoagouti young (Table 3). In an earlier study, the respective frequencies of pseudoagouti young produced by this reciprocal cross were 17% and 34% (WOLFF 1971). These data suggest that the background genome of the dam also influences the direction of differentiation of the A^{vy}/a zygote.

Comparison of the data in Table 1 with those in Table 4 also illustrates vividly the importance of the phenotypic expression of the background genome of the dam in influencing the phenotypic differentiation of the A^{vy}/a genotype. Although the proportion of pseudoagouti young produced by nonyellow females of the YS, VY, and AT strains differs markedly (Table 1), the proportion of

TABLE 3

Frequency of pseudoagouti A^{vy}/a *offspring in a reciprocal cross*

Cross	Genotypes Dam	Sire	A^{vy}/a offspring Pseudoagouti %	N	Mottled yellow %	N
VY/Wf × YS/ChWf	a/a	A^{vy}/a	9.6	22	90.4	208
YS/ChWf × VY/Wf	a/a	A^{vy}/a	20.7	25	79.3	96

TABLE 4

Proportion of pseudoagouti mice among A^{vy}/a *offspring in three* F_1 *hybrids*

		$A^{vy}/-$ offspring			
		Pseudoagouti		Mottled yellow	
Dam	Sire	%	N	%	N
C57BL/6J-a/a	YS/ChWf-A^{vy}/a	13.4	37	86.6	238
C57BL/6J-a/a	VY-Wf-A^{vy}/a	14.6	33	85.4	193
C57BL/6J-a/a	AT/Wf-A^{vy}/a^t	12.9	38	87.1	257

this A^{vy}/a phenotype produced by C57BL/6J dams is the same regardless of the strain of the yellow males to which they were mated.

The AM/Wf strain provides more evidence that the pseudoagouti phenotype, whether A^{vy}/a^m or a^m/a^m genetically, is highly dependent for its differentiation on the background genome, as well as on the agouti-locus phenotype of the mother. During the first generations of inbreeding of this strain, pseudoagouti offspring were produced with reasonable regularity, but beyond the 10th generation of inbreeding pseudoagouti offspring ceased to appear. Analysis of the breeding data for the first ten generations of sib matings indicated a frequency of about 12% pseudoagouti young among all offspring of A^{vy}/a^m dams mated by a^m/a^m sires, whereas among the reciprocal types of matings 20% of the offspring were of the pseudoagouti phenotype.

Although appearance of pseudoagouti animals of either genotype in the AM strain stopped many generations ago, mating either A^{vy}/a^m or a^m/a^m males by C57BL/6J females resulted in the appearance of a considerable proportion of animals of this phenotype. Breeding tests of 37 pseudoagouti animals from this F_1 hybrid indicated that 13.5% (5/37) were A^{vy}/a and 86.5% (32/37) were a^m/a genotypically. These proportions are in remarkably good agreement with those determined by breeding tests during the early generations of inbreeding. At that time 103 pseudoagouti animals were tested, 21% were found to be A^{vy}/a^m and 79% were a^m/a^m (WOLFF 1971). It appears that the $a^m/$— zygotes differentiate into the pseudoagouti phenotype with a higher frequency than $A^{vy}/$— zygotes.

TABLE 5

Frequency of pseudoagouti mice of two genotypes in a reciprocal cross

Strain and genotype		Offspring							
		Pseudoagouti				Mottled			
		A^{vy}/a		a^m/a		A^{vy}/a		a^m/a	
Dam	Sire	%	N	%	N	%	N	%	N
AM/Wf-a^m/a^m	C57BL/6J-a/a	—	—	0	0	—	—	100	366
C57BL/6J-a/a	AM/Wf-a^m/a^m	—	—	29.5	125	—	—	70.5	299
AM/Wf-A^{vy}/a^m	C57BL/6J-a/a	0	0	0	0	46.2	171	53.8	199
C57BL/6J-a/a	AM/Wf-A^{vy}/a^m		18.8%	127*		47.7	314	35.3	232

* A^{vy}/a and a^m/a combined.

In Table 5 the data on the frequencies of pseudoagouti offspring from the C57BL/6J × AM/Wf matings are tabulated. C57BL/6J × a^m/a^m matings produced 29% pseudoagouti young. C57BL/6J × A^{vy}/a^m matings produced only about 19% pseudoagouti offspring. Based on the results of the breeding tests mentioned above, it can be calculated that among the F_1 hybrid offspring from A^{vy}/a^m sires, 5.1% of all A^{vy}/a young and 32% of all a^m/a offspring were pseudoagouti (Table 6).

Reciprocal matings of A^{vy}/a^m or a^m/a^m females by C57BL/6J males produced no pseudoagouti offspring (Table 5), again emphasizing the importance of the strain genome of the dam in the phenotypic expression of these genotypes.

DISCUSSION

The earliest report of an apparent maternal influence on the development of zygotes carrying mutations at the agouti locus is that of ROBERTSON (1941). His data indicated that lethal yellow (A^y/A^y) homozygous embryos developed somewhat further in agouti (A/A) than in yellow (A^y/A) mothers before dying. He concluded "that the environmental effect, which acts through the maternal endometrium to influence the development of the homozygous yellow mouse embryos, must be due to the action of the A^y locus in the maternal genotype."

In the YS/ChWf strain, embryonic survival was lower in yellow (A^y/a) mothers than in black (a/a) dame, and there was a deficiency of yellow mice at weaning age; however, these may be strain-specific phenomena (WOLFF and BARTKE 1966). In that report it was postulated that the same factor that induces the death of A^y/A^y embryos may also decrease the viability of the heterozygous A^y/a embryos. Thus, the proportion of A^y/a embryos that die may be determined by the intraoviductal or intrauterine milieu of the mother. These appeared to be influenced greatly by the yellow A^y/a phenotype, as well as by the strain genome.

GLASS (1963) reported that, in the mouse, serum-like molecules of maternal origin could be detected in the cytoplasm of preovulatory ovarian oocytes, in ootid cytoplasm and in embryonic blastomeres at all preimplantation stages.

TABLE 6

Distribution of A^{vy}/a *and* a^m/a *genotypes among pseudoagouti mice*

Strain and genotype		Offspring							
		Pseudoagouti				Mottled			
		A^{vy}/a		a^m/a		A^{vy}/a		a^m/a	
Dam	Sire	%	N	%	N	%	N	%	N
C57BL/6J-a/a	AM/Wf-a^m/a^m	—	—	29.5	125	—	—	70.5	299
C57BL/6J-a/a	AM/Wf-A^{vy}/a^m		18.8%	127*		47.7	314	35.3	232
	Calculated†:	5.1	17	32	110				

* A^{vy}/a and a^m/a combined.

† Based on breeding tests of pseudoagouti mice:

$a^m/a = 32/37 = 86.5\%$.

$A^{vy}/a = 5/37 = 13.5\%$.

More recently, the same investigator reported that, following intravenous injection of bovine plasma albumin into pregnant mice, intraembryonic localization of the antigen differed according to developmental stage of the embryo (GLASS and HANSON 1975).

These findings suggest that the metabolic characteristics of the dam can influence, in a major and direct manner, the development and differentiation of the embryos. The relative availability to the embryos of diverse maternal molecular species may differ considerably between yellow and nonyellow mothers of the same inbred strain or F_1 hybrid and thus could influence the differentiation of the more labile phenotypes in different directions.

The death of homozygous lethal yellow (A^y/A^y) embryos about the time of implantation (EATON and GREEN 1963) indicates that the genes at the agouti locus are activated at a very early stage of development. This may occur before or during the second cleavage division according to the observations of PEDERSEN and SPINDLE (1976). These investigators noted a delay in the transition from the two-cell to the four-cell stage in cultured A^y/A^y embryos. Accordingly, the alternative differentiation of A^{vy}/a or $a^m/-$ embryos into either mottled or pseudoagouti phenotypes most probably should be viewed as resulting from a differential response of the phenotypically labile A^{vy}/a or $a^m/-$ zygotes or embryos to the metabolic milieu of the dam's oviduct or uterus.

Of special interest is the maternal influence on the differentiation of the metabolic regulatory systems and on susceptibility to tumor formation of the A^{vy}/a phenotypes. As reported earlier (WOLFF 1971; WOLFF and PITOT 1972), the mottled yellow and pseudoagouti A^{vy}/a mice differ considerably in their rate of weight gain, susceptibility to spontaneous hepatoma formation, rate of growth of solid tumors from implanted and allogeneic ascites cells, and other physiological characteristics.

The concomitant effects of the A^{vy}/a genotype on hair-pigment pattern and on metabolic regulation, as exemplified by obesity and enhanced normal and neoplastic growth, suggest a close relationship between these seemingly disparate processes. The apparent linkage among them is emphasized by the concomitant alterations of the hair pigment pattern and the effects on obesity and neoplastic growth—both in the direction of "normality"—in the pseudoagouti A^{vy}/a phenotype.

The apparent very close relationship between the endoplasmic reticulum (E. R.) of the melanocyte and the formation of melanosomes described most recently by SAKURAI, OCHIAI and TAKEUCHI (1975) suggests a possible common denominator for the simultaneous effects of the several "yellow" alleles at the agouti locus (DICKIE 1969) on hair-pigment pattern and metabolic regulation.

The studies of MOYER (1966) and SAKURAI, OCHIAI and TAKEUCHI (1975) suggest that the agouti locus controls one (or more) factors in the hair bulb that determines the ultrastructure of the melanosomes produced by the melanocytes. Melanosomes that contain eumelanin are rod shaped and contain parallel cross-linked filaments. Melanosomes that contain phaeomelanin are spherical and contain a disorganized filamentous matrix. Both types of melanosomes are found in melanocytes from genetically agouti ($A/-$) mice. In some melanocytes, phaeomelanosomes were found to have some continuity with the E. R. (SAKURAI, OCHIAI and TAKEUCHI 1975). A similar observation has been reported for eumelanosomes in human melanoma cells (MAUL 1969). SAKURAI, OCHIAI and TAKEUCHI (1975) stated that the structure of the precursors of phaeomelanosomes and eumelanosomes appear morphologically distinct. Thus, it appears that the agouti locus alters the microenvironmental conditions that influence melanosome structure and that the switch between eumelanin and phaeomelanin synthesis results from changes in melanosome structure induced by cyclical changes in the microenvironment of the melanocytes.

The apparent derivation of the melanosomes from the E.R. suggests that it may be an important focus of action of the postulated regulatory factor (WOLFF 1971) controlled by the agouti locus. Since the E. R. is a major site of protein synthesis, alteration of a parameter that affects one or more of its functions could have major effects throughout the metabolic network.

These considerations suggest that the coat-color patterns induced by different agouti-locus alleles are visible markers that reflect metabolic changes induced either locally (POOLE and SILVERS 1976) or systemically, as in the case of the "yellow" alleles. It seems possible that all of these effects may result from direct or indirect alteration of certain characteristics or functions of the E. R. by the action of the agouti alleles.

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