INFLUENCE OF BACKGROUND GENOME ON ENZYMATIC CHARACTERISTICS OF YELLOW ($A^y/-$, $A^{vy}/-$) MICE[1]

GEORGE L. WOLFF[2] AND HENRY C. PITOT

The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania and McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, Wisconsin

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ABSTRACT

Identification of the fundamental polypeptide difference between yellow ($A^y/-$, $A^{vy}/-$) and non-yellow mice is important for biomedical research because of the influence of the yellow genotype on normal and neoplastic growth and obesity. The complexity of the "yellow mouse syndrome" makes attainment of this objective dependent on the separation of those pleiotropic enzyme differences which are secondary, and depend on the background genome, from those which are primary, and depend primarily on the agouti locus genotype.——Four of nine hepatic enzyme activities assayed simultaneously differed between eight-week-old yellow ($A^y/-$, $A^{vy}/-$) and non-yellow ($A/-$, a/a) male inbred and F_1 hybrid mice. Among these four, only cytoplasmic malic enzyme activity was elevated in all yellow mice, as compared with the non-yellow sibs, regardless of background genome. Glucokinase, serine dehydratase, and tyrosine α-ketoglutarate transaminase activities were also changed in yellow mice, but these alterations depended on the background genome.——The ratio of malic enzyme activity to citrate-cleavage enzyme activity, possibly related to the altered fat metabolism of yellow mice, was influenced by background genome as well as by the yellow genotype.——Significant deviations of enzyme activities from mid-parent values among F_1 hybrids were associated with particular background genomes; the number of such deviations was larger among yellow mice than among non-yellows and this difference was greater among C3H F_1 hybrids than among C57BL/6 F_1 hybrids.

DEPENDENCE of the phenotypic expression of mutant genes on the background genome in the mammal is well illustrated by the physiologic and enzymatic characteristics of yellow ($A^y/-$, $A^{vy}/-$) mice. While this aspect of gene expression has been documented before, e.g. the dominant spotting (W) alleles (RUSSELL and LAWSON 1959), muscular dystrophy (dy) (RUSSELL *et al.* 1962), it assumes additional importance for biomedical research because the

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[2] Present address: Division of Special Studies, National Center for Toxicological Research, Jefferson, Arkansas 72079.

"yellow" alleles at the agouti locus exert such a pronounced influence on metabolic regulation, as reflected by the enhancement of normal and neoplastic growth and excess fat deposition in yellow mice. Identification of the polypeptide under direct control of the agouti locus would aid immeasurably in definition of the alterations in the metabolic network, induced by the mutant polypeptide, which result in the complex pleiotropic "yellow mouse" syndrome. The very complexity of the relations among the considerable number of physiologic and biochemical differences which exist between two organisms as different metabolically as the yellow and non-yellow litter mates has been a major obstacle to identification of this polypeptide. This problem is aggravated by the apparent dependence of many of the pleiotropic effects on the residual background genome. In view of these difficulties, it was deemed advisable to determine the relative importance of the background genome and of the A^{y} or A^{vy} genes *per se* to establishment of the metabolic differences between the yellow and non-yellow sibs.

Previous studies on the influence of these "yellow" genes and the strain background on diverse physiological parameters (WOLFF 1965, 1970a,b, 1971; WOLFF and REICHARD 1970; WOLFF and FLACK 1971) were undertaken with a view to identifying the most promising experimental system to use for enzymatic studies. Present and future enzymatic studies are aimed toward identifying the primary polypeptide affected by the agouti locus. The basic criterion of degree of relationship of a particular enzyme activity to the primary effect of the "yellow" genes is the constancy of the difference between the yellow and non-yellow sibs in the specific parameter measured regardless of physiologic conditions or genetic background.

The present report details the results provided by exploratory assays of the catalytic capacities of nine key enzymes in livers obtained from eight-week-old male mice from four inbred strains and four F_1 hybrids in which yellow and non-yellow mice segregate.

The enzymes selected for study were representative enzymes involved in gluconeogenesis from amino acids, several being relatively specific for liver such as serine dehydratase and histidase. Enzymes of glycogen metabolism (glucokinase), glucose and fat metabolism (hexokinase, malic enzyme, etc.) represented two other major nutritional pathways. Thus, rate-limiting steps in a number of major metabolic pathways were examined.

Influence of strain genome on physiological expression of the yellow genotypes: In order to place the present report in the proper perspective, a brief review of the influence of strain genome on some physiologic characteristics of yellow mice may be useful. In this regard, the strain and tissue specificity of the enhancement of neoplastic growth by these mutant genes is especially significant because of its potential use in identifying specific characteristics of cell and tissue metabolism which favor neoplastic growth. Thus, among F_1 hybrids from matings of the lung tumor-susceptible A/He strain with the Y/He strain, yellow A^{y}/a sibs developed more lung tumors than the non-yellow mice (MORGAN 1950); however, F_1 hybrids from matings of the hepatoma-susceptible C3HfB/He strain with the YBR/He strain were quite resistant to the formation of pulmonary tumors but

were highly susceptible to hepatoma formation (HESTON and VLAHAKIS 1966). Among these F_1 hybrids, the yellow A^y/A mice had more hepatomas per liver than their agouti A/a sibs; no difference between these yellow and agouti mice was observed in the distribution of the few lung tumors which developed. Even in inbred strains with relatively low spontaneous hepatoma incidence the A^y and A^{vy} genes enhance tumor formation (WOLFF and PITOT 1972b). This enhancement of strain- and tissue-specific neoplastic growth may result from alteration of the metabolic characteristics of the susceptible tissue, just as the alteration of the hair bulb environment by agouti locus alleles induces changes in hair pigment synthesis by the follicular melanocytes (SILVERS and RUSSELL 1955; SILVERS 1958a,b).

The enhancement of normal growth by these "yellow" genes also is dependent on the strain background. Among inbred YS/ChWf strain mice as well as among F_1 hybrids from matings of YS mice with C3H, C57BL/6, and BALB/c strain mice, the difference in liver weight between yellow and non-yellow mice did not become apparent until *after* four weeks of age (WOLFF 1970). In contrast, among inbred VY/Wf strain mice and among F_1 hybrids from matings of VY mice with mice of the same three strains, the difference in liver wet weight between yellow and non-yellow mice was already noticeable at four weeks of age (WOLFF 1970).

Enhancement of fat deposition in yellow mice also depends on the background genome. Inbreeding has been reported to reduce the obesity of yellow mice which was restored to higher levels in F_1 hybrids (DICKIE and WOOLLEY 1946; FENTON and CHASE 1951; MORGAN 1950). WOLFF and REICHARD (1970) found that the relative excess in body weight of yellow mice at three months of age as compared to their black sibs depended on the strain of the mother as follows:

	Dam	Sire	Percent excess body weight of yellow offspring in relation to their black sibs
Inbred	YS-a/a	YS-A^y/a	+ 26%
F_1 hybrid	YS-a/a	VY-A^{vy}/a	+ 24%
Inbred	VY-a/a	VY-A^{vy}/a	+ 45%
F_1 hybrid	VY-a/a	YS-A^y/a	+ 35%

HESTON and VLAHAKIS (1966) reported similar maternal effects with respect to body weight among yellow and non-yellow F_1 hybrid mice from YBR/He × C3HfB/He and reciprocal matings. While possible differences in lactating ability may be the immediate causative "maternal effect", such physiological differences, of course, reflect fundamental differences between the strain genomes.

The background genome also influences the response of serum insulin concentration to growth of allogeneic Sarcoma 37 cells in inbred YS and VY strain mice and their F_1 hybrids (WOLFF and REICHARD 1970; WOLFF 1971). Hormone concentration among inbred and F_1 hybrid mice which had YS-a/a mothers did not respond to Sarcoma 37 growth; however, inbred and F_1 hybrid mice with VY-a/a mothers exhibited several-fold increases in hormone concentration after eight days of tumor cell growth. These differences in hormonal response were not

directly related to body weights since these were the same for the black (a/a) and agouti (A^{vy}/a) mice from both crosses (WOLFF 1971).

Influence of the strain genome is also expressed in the coat color effect of the "viable yellow" A^{vy}/a genotype (WOLFF 1971). This genotype induces a continuum of coat color phenotypes ranging from completely yellow through various degrees of yellow, black, and agouti mottling to completely agouti animals. These agouti mice are indistinguishable from wild type $A/-$ mice except by breeding tests. Agouti $A^{vy}/a \times$ black a/a matings produce 50% mottled yellow: 50% black offspring, and the frequency of agouti A^{vy}/a offspring is not higher than among mottled yellow $A^{vy}/a \times$ black a/a matings. The agouti A^{vy}/a mice appear with a frequency which seems to be strain-dependent (WOLFF 1971). Among A^{vy}/a offspring from VY-$a/a \times$ YS-A^{vy}/a matings, 17% were of the agouti phenotype, whereas among A^{vy}/a offspring from YS-$a/a \times$ VY-A^{vy}/a matings, the frequency of agouti offspring was twice as high (WOLFF 1971). Except for the Y chromosome, mice from both types of mating were genetically identical. While the phenotypic differences between yellow and agouti A^{vy}/a mice apparently are not based on genetic differences, the *frequency* of the agouti phenotype seems to be influenced by a maternal effect dependent on the metabolic expression of the strain genome.

MATERIALS AND METHODS

Strains and F_1 hybrids: The following inbred strains and F_1 hybrids were used (agouti locus genotypes of offspring noted in parentheses): C3H/HeNIcr (A/A), C57BL/6JNIcr (a/a), YS/ChWf (A^y/a, a/a), VY/Wf (A^{vy}/a, a/a), C3H $\times$ YS (A^y/A, A/a), C3H $\times$ VY (A^{vy}/A, A/a), C57BL/6 $\times$ YS (A^y/a, a/a), C57BL/6 $\times$ VY (A^{vy}/a, a/a). Agouti locus phenotypes: $A^y/- =$ clear yellow, $A^{vy}/- =$ mottled yellow, $A/- =$ agouti, $a/a =$ non-agouti. (In the strain combinations used in this work, all non-agouti animals were black).

The YS strain was started in 1948 when CHASE introduced recessive spotting into a yellow stock from The Jackson Laboratory and then crossed the stock with C57BL mice (STAATS 1972). Breeding stock was supplied by DR. CHASE in 1959; the YS/ChWf substrain has been maintained by sibmating since then.

The VY strain was started in 1962 by mating a third backcross generation C57BL/6J-A^{vy} male with two first backcross generation C57BL/6J-A^{vy} sisters (STAATS 1972). The offspring were sibmated. Sibmating has been used exclusively in development of the strain. The A^{vy} mutation arose in the C3H/Di strain (DICKIE 1962). Therefore the VY strain contains between 6.25% and 25% of the C3H/Di genome.

Both the YS and VY strains are maintained by mating heterozygous yellow mice with their black sibs.

Animal care: Two to eight males were kept together from weaning to eight weeks of age in stainless steel shoe-box type cages with sterilized white pine shavings as bedding. Old Guilford Mouse and Rat Breeder Pellets (7.5% fat) and hyperchlorinated acidified drinking water were available *ad libitum*. Room temperature was maintained at 76 $\pm$ 2°F with 45–55% relative humidity; 11 hr of fluorescent light were provided daily. Cages were washed weekly; water bottles, twice weekly.

Tissues: The animals were not fasted and were weighed just before decapitation. Livers were removed immediately after exsanguination of each animal, cooled over ice while the gall bladder was excised, and then frozen with solid CO_2. All livers were processed in the morning and shipped by air to the McArdle Laboratory in solid CO_2 on the same day. They were weighed prior to homogenization.

Enzyme assays: Enzyme activities were assayed by an automated instrument described

previously (PITOT and PRIES 1964; PITOT, WRATTEN and POIRIER 1968). All assays were carried out on the high-speed supernatant (105,000 × g for 90 min), with the exception of ornithine transaminase. This enzyme was assayed in the whole homogenate by the method of PERAINO and PITOT (1963). In all instances the assays, performed on up to 40 mg wet weight of tissue, demonstrated linearity with time up to 30 min.

Statistical significance of differences was determined by analyses of variance (SOKAL and ROHLF 1969) or by *t* tests for groups of equal and unequal size (SNEDECOR 1946).

RESULTS

The catalytic capacity of a tissue with respect to a single enzyme has been defined as "the specific enzyme activity observed with assays done under conditions of zero order kinetics with neither substrate nor cofactors being limiting" (WOLFF and PITOT 1972a). We have explained that "it is an estimate of the potential specific activity of the enzyme in a tissue at a particular point in the circadian cycle and bears no direct relation to the actual *in vivo* activity rates" (WOLFF and PITOT 1972a). The primary objective of the present analysis was to determine which, if any, of the enzyme capacities assayed maintained a constant relationship either with a particular agouti locus genotype or with a particular strain genome. Accordingly, the variation of catalytic capacities of the hepatic enzymes with inbred strain genome is described. In addition, the alterations in these enzyme capacities induced by changes in the agouti locus genotype as well as by changes in the background genome through hybridization are enumerated.

Hepatic enzyme capacities in inbred strains—Table 1

Hexokinase: The catalytic capacity of this enzyme was only about 50% as great in C3H males as in C57BL/6 males ($P = .01$). Black YS and VY males resembled the C57BL/6 males but yellow YS and VY males had somewhat greater capacity than their black sibs ($P = .01$–$.025$).

Glucokinase: Catalytic capacity was similar in all inbred strains. No effect of the yellow phenotypes was noted.

Phosphogluconate dehydrogenase: Capacity was highest in the C57BL/6 and VY males, slightly lower in YS males ($P = .025$–$.05$), and lowest in C3H males ($P = .01$–$.02$). Capacity was slightly increased in the yellow phenotypes of both strains ($P = .005$–$.01$).

Citrate-cleavage enzyme: Capacity was highest in VY males, lower in YS males ($P < .001$), and lowest among C3H and C57BL/6 males which were similar. No effects of the yellow genotypes were noted.

Malic enzyme: Capacity was greatest in C3H and C57BL/6 males which resembled each other. VY males had lower capacity than these strains, but it was greater than that in YS males ($P = .001$–$.005$). In the yellow phenotypes, capacity was increased ($P = .001$–$.005$) to approximately the levels observed in C3H and C57BL/6 livers; the strain difference between the VY and YS mice was retained in the yellow phenotypes.

Serine dehydratase: No differences in capacity due to strain genome or agouti locus genotype were noted.

TABLE 1

Catalytic capacities of hepatic enzymes and body weights of male mice of four inbred strains at eights weeks of age

Enzyme and coat color phenotype	Strains			
	C3H/HeNIcr	C57BL/6JNIcr	VY/Wf	YS/ChWf
Hexokinase				
Yellow*			41.7 ± 5.9 (6)	40.1 ± 7.1 (5)
Non-yellow*	18.5 ± 2.6 (10)†	32.2 ± 3.5 (7)	33.4 ± 4.5 (5)	26.9 ± 4.6 (8)
Glucokinase				
Yellow			94.6 ± 6.6 (7)	102.9 ± 11.3 (10)
Non-yellow	108.7 ± 11.6 (10)	99.8 ± 8.0 (10)	75.9 ± 9.8 (8)	87.0 ± 6.2 (7)
Phosphogluconate dehydrogenase				
Yellow			217.2 ± 10.4 (10)	191.7 ± 13.5 (10)
Non-yellow	142.6 ± 4.6 (10)	191.6 ± 10.2 (10)	184.7 ± 6.8 (10)	166.0 ± 7.2 (10)
Citrate-cleavage enzyme				
Yellow			272.2 ± 18.4 (10)	196.0 ± 14.5 (10)
Non-yellow	145.3 ± 8.9 (10)	126.6 ± 9.7 (10)	240.0 ± 8.0 (10)	190.6 ± 6.3 (10)
Malic enzyme				
Yellow			394.7 ± 39.6 (10)	287.1 ± 26.1 (10)
Non-yellow	365.4 ± 28.6 (6)	341.0 ± 35.4 (10)	276.6 ± 27.8 (10)	211.9 ± 18.2 (10)

Serine dehydratase				
Yellow			2209.7 ± 119.2 (10)	1608.6 ± 161.5 (10)
Non-yellow	1934.2 ± 100.1 (10)	1907.2 ± 192.3 (10)	2275.5 ± 243.8 (10)	2116.9 ± 236.7 (10)
Histidase				
Non-yellow			39.1 ± 3.5 (9)	17.7 ± 0.7 (10)
Yellow	42.2 ± 1.7 (10)	65.3 ± 6.5 (10)	35.7 ± 3.0 (10)	19.1 ± 1.4 (10)
Tyrosine α-ketoglutarate transaminase				
Yellow			113.5 ± 9.3 (10)	81.7 ± 7.5 (7)
Non-yellow	72.9 ± 5.6 (8)	52.2 ± 3.6 (8)	101.6 ± 7.9 (10)	85.9 ± 9.5 (10)
Ornithine transaminase				
Yellow			41.0 ± 5.6 (10)	36.0 ± 2.1 (10)
Non-yellow	87.0 ± 5.0 (10)	144.7 ± 10.8 (10)	41.3 ± 4.0 (10)	31.6 ± 1.7 (3)
Body weights				
Yellow			25.6 ± 0.7 (10)	23.9 ± 0.6 (10)
Non-yellow	26.4 ± 0.5 (10)‡	24.2 ± 0.4 (10)	21.9 ± 0.5 (10)	22.8 ± 0.3 (10)

* Agouti locus genotypes:

Yellow			A^{vy}/a	A^{y}/a
Non-yellow	A/A	a/a	a/a	a/a

† Mean μmoles product/gm liver/hr. ± S.E. (N); (N) = Number of mice.
‡ Mean gm ± S.E. (N); (N) = Number of mice.

Histidase: C57BL/6 males had the highest catalytic capacity, C3H and VY males had less capacity, and YS males had approximately 50% as much capacity as the C3H and VY mice. No effects of the yellow genotypes were observed.

Tyrosine α-ketoglutarate transaminase: VY males had the highest catalytic capacity. This was slightly greater than that found in YS males ($P = .01$). C3H males resembled YS males in this enzyme capacity. C57BL/6 males had the lowest mean value but this was not statistically different from the levels in C3H males. No differences between the capacities of yellow and black mice, VY or YS, were noted.

Ornithine transaminase: C57BL/6 males had much greater capacity than C3H mice ($P < .001$). C3H mice had about twice as much catalytic capacity as VY and YS mice. There were no differences between YS and VY mice, either yellow or black, and no effect of the yellow genotypes was noted.

Hepatic Enzyme Capacities in F_1 Hybrids—Table 2

Hexokinase: No differences in catalytic capacity among any of the non-yellow F_1 hybrid classes were noted. Yellow C57BL/6 F_1 hybrids had increased capacity compared with their non-yellow sibs ($P = .01$–$.025$); such an effect was absent among C3H F_1 hybrids.

Glucokinase: C3H F_1 hybrids exhibited greater catalytic capacity than C57BL/6 F_1 hybrids ($P < .001$). Only among VY F_1 hybrids did the yellow mice have greater capacity than non-yellow males ($P = .001$–$.005$).

Phosphogluconate dehydrogenase: Among VY F_1 hybrids, capacity was greater in C57BL/6 × VY than in C3H × VY livers. No differences between the YS F_1 hybrids were found. Yellow C3H F_1 hybrids had somewhat greater catalytic capacity than their non-yellow sibs ($P = .01$–$.025$); no such difference was observed among the C57BL/6 F_1 hybrids.

Citrate-cleavage enzyme: Catalytic capacity was similar in all F_1 hybrid categories except in C3H × VY mice, in which it was slightly lower. No marked effects of the yellow genotypes were noted.

Malic enzyme: In all F_1 hybrids catalytic capacity was greater in the yellow than in the non-yellow animals ($P < .001$). There were no differences between C3H and C57BL/6 F_1 hybrids nor between YS and VY F_1 hybrids.

Serine dehydratase: No differences in catalytic capacity among the various F_1 hybrid classes were observed. Capacity was decreased in yellow C3H F_1 hybrids ($P = .001$–$.01$) but not in yellow C57BL/6 F_1 hybrids in comparison with the respective non-yellow males.

Histidase: Catalytic capacity was lower among C3H F_1 hybrids than in the C57BL/6 F_1 hybrids ($P < .001$). It was lower among YS F_1 hybrids than among VY F_1 hybrids ($P = .001$–$.005$). No differences between yellow and non-yellow mice were observed.

Tyrosine α-ketoglutarate transaminase: There were no differences in catalytic capacity among the non-yellow mice of the various classes. In all categories yellow mice had greater catalytic capacity than their non-yellow sibs ($P < .001$).

Ornithine transaminase: No differences in catalytic capacity due to strain or agouti locus genotype were noted.

Correlations: Three pairs of catalytic capacities had significant correlation coefficients in the C3H F_1 hybrids (all genotypes combined) but not in the C57BL/6 F_1 hybrids. Glucokinase and phosphogluconate dehydrogenase capacities were positively correlated ($r = 0.40$; d.f. $= 38$; $P = .01$). Tyrosine α-ketoglutarate transaminase capacity was negatively correlated with histidase capacity ($r = -0.38$; d.f. $= 38$; $P = .01$–$.05$) and with serine dehydratase capacity ($r = -0.45$; d.f. $= 38$; $P = .001$–$.01$).

DISCUSSION

Four of the hepatic enzyme capacities assayed differed between yellow and non-yellow mice. Of these four, cytoplasmic malic enzyme capacity showed the closest relation to the primary effect of the A^y and A^{vy} genes, since all yellow mice, regardless of genetic background, had higher values than comparable non-yellows. The mechanism of this effect of the yellow genotypes on malic enzyme capacity is not clear, since the structural locus for the enzyme (*Mod-1*) is located on chromosome 10 (LG II) (HUTTON and RODERICK 1970), while the agouti locus is located on chromosome 2 (LG V) (GREEN 1966).

The catalytic capacities of the other three enzymes differed between yellow and non-yellow mice only in the presence of particular background genomes as follows:

	Inbred mice	F_1 hybrid mice
Glucokinase	Yellow = black	Yellow VY F_1 > Non-yellow VY F_1 Yellow YS F_1 = Non-yellow YS F_1
Serine dehydratase	Yellow = black	Non-yellow C3H F_1 > Yellow C3H F_1 Non-yellow C57BL/6 F_1 = Yellow C57BL/6 F_1
Tyrosine α-ketoglutarate transaminase	Yellow = black	Yellow > Non-yellow

Deviations of catalytic capacities from the mid-parent values among the F_1 hybrids also appear to depend on background genome (Table 3). For each enzyme, these deviations from mid-parent values were always in the same direction, i.e., either positive or negative, in all genotypic categories in which they occurred. For example, serine dehydratase capacity was decreased while glucokinase capacity was increased. Glucokinase capacity was greater than expected only among C3H F_1 hybrids; their body weights were also greater than expected.

It is interesting to note that the number of significant deviations of catalytic capacities from the mid-parent values was greater among yellow mice than among non-yellows (13:7) and that this difference was more pronounced among the C3H F_1 hybrids (8:4) than among the C57BL/6 F_1 hybrids (5:3). The significance of these observations as well as the significance of the correlations found in three sets of enzymes in the C3H F_1 hybrids, but not among the C57BL/6 F_1 hybrids, is not clear and requires more detailed investigation.

TABLE 2

Catalytic capacities of hepatic enzymes, liver weights, and body weights of male mice of four F_1 hybrids at eight weeks of age

Enzyme and coat color phenotype	F_1 hybrids			
	C3H × VY	C3H × YS	C57BL/6 × VY	C57BL/6 × YS
Hexokinase				
Yellow*	20.5 ± 2.9 (7)†	27.1 ± 5.0 (7)	43.7 ± 2.7 (10)	40.7 ± 4.7 (10)
Non-yellow*	20.5 ± 6.1 (9)	26.1 ± 4.8 (10)	28.9 ± 3.0 (7)	26.5 ± 4.3 (10)
Glucokinase				
Yellow	177.8 ± 17.2 (10)	142.4 ± 10.1 (10)	101.5 ± 6.1 (9)	102.7 ± 12.8 (10)
Non-yellow	122.8 ± 10.0 (10)	133.5 ± 12.8 (10)	77.2 ± 6.7 (8)	83.9 ± 6.6 (8)
Phosphogluconate dehydrogenase				
Yellow	142.4 ± 6.5 (9)	158.7 ± 6.8 (10)	172.5 ± 8.7 (8)	156.5 ± 19.0 (10)
Non-yellow	103.7 ± 8.3 (10)	136.0 ± 5.0 (10)	163.1 ± 6.8 (10)	152.1 ± 14.1 (10)
Citrate-cleavage enzyme				
Yellow	154.8 ± 12.1 (10)	185.3 ± 9.2 (10)	202.4 ± 21.1 (10)	192.1 ± 12.4 (10)
Non-yellow	156.8 ± 9.4 (10)	188.2 ± 10.4 (10)	192.9 ± 19.6 (9)	156.3 ± 5.8 (10)
Malic enzyme				
Yellow	392.2 ± 29.2 (10)	477.0 ± 39.1 (10)	508.5 ± 33.2 (10)	333.0 ± 15.0 (10)
Non-yellow	299.0 ± 18.4 (10)	295.3 ± 24.7 (8)	385.8 ± 26.4 (10)	248.6 ± 15.3 (9)

Serine dehydratase				
Yellow	1274.4 ± 65.6 (8)	1438.6 ± 126.0 (10)	1672.8 ± 124.6 (10)	1437.5 ± 72.6 (10)
Non-yellow	1661.0 ± 58.1 (10)	1832.9 ± 119.7 (10)	1611.3 ± 90.3 (10)	1513.3 ± 62.3 (10)
Histidase				
Yellow	29.3 ± 2.7 (10)	22.8 ± 1.6 (10)	42.7 ± 1.5 (10)	36.7 ± 1.8 (10)
Non-yellow	33.4 ± 1.2 (10)	28.3 ± 1.0 (10)	43.8 ± 3.2 (10)	36.9 ± 1.9 (10)
Tyrosine α-ketoglutarate transaminase				
Yellow	112.6 ± 10.2 (10)	113.9 ± 10.2 (10)	92.8 ± 8.0 (10)	85.2 ± 5.4 (10)
Non-yellow	79.7 ± 6.1 (10)	77.8 ± 7.0 (10)	68.6 ± 7.3 (10)	70.6 ± 4.4 (10)
Ornithine transaminase				
Yellow	47.2 ± 3.5 (10)	50.6 ± 5.4 (10)	40.9 ± 3.1 (9)	37.9 ± 3.3 (10)
Non-yellow	50.1 ± 5.5 (10)	46.5 ± 2.6 (9)	38.0 ± 2.2 (8)	49.1 ± 2.6 (10)
Body weight				
Yellow	34.7 ± 2.3 (10)‡	33.4 ± 0.9 (10)	26.8 ± 0.5 (10)	24.4 ± 0.8 (10)
Non-yellow	29.3 ± 0.7 (10)	28.8 ± 0.5 (10)	23.1 ± 0.3 (9)	23.6 ± 0.6 (10)
Liver weight				
Yellow	2.04 ± .15 (10)‡	1.93 ± .08 (10)	1.59 ± .07 (10)	1.40 ± .06 (10)
Non-yellow	1.65 ± .07 (10)	1.47 ± .05 (10)	1.29 ± .05 (9)	1.26 ± .04 (10)

* Agouti locus genotypes:

Yellow	A^{vy}/A	A^{y}/A	A^{vy}/a	A^{y}/a
Non-yellow	A/a	A/a	a/a	a/a

† Mean μmoles product/gm liver/hr. ± S.E. (N); (N) = Number of mice.
‡ Mean gm ± S.E. (N); N = Number of mice.

TABLE 3

Deviations of hepatic enzyme capacities and body weights from mid-parent values among F_1 hybrid male mice at eight weeks of age

	C3H × VY		C3H × YS		C57BL/6 × VY		C57BL/6 × YS	
	Y†	N†	Y	N	Y	N	Y	N
Glucokinase	+73%***	+30%§	+35%‖	+34%‖				
Phosphogluconate dehydrogenase	—21%‖	—37%***						
Citrate cleavage enzyme	—26%*							
Malic enzyme			+51%***		+38%**			
Serine dehydratase	—38%***	—21%§			—19%§	—23%§		—25%§
Histidase	—28%**							
Tyrosine transaminase			+48%**				+29%§	
Ornithine transaminase					—56%¶	—59%¶	—58%¶	‡
Body weights	+34%***	+21%***	+33%***	+17%***				

† Y = yellow phenotypes ($A^{vy}/-$ or $A^{y}/-$); N = non-yellow phenotypes ($A/a, a/a$)
* P = .05
** P = .01–.001
§ P = .02–.05
*** P < .001
‖ P = .01
‡ Insufficient data
¶ P = .01–.02

One of the best-known characteristics of yellow mice is their predisposition to obesity. The enzymatic basis for this characteristic is unknown. Among the hepatic enzymes assayed, malic enzyme and citrate-cleavage enzyme are both intimately involved in fatty acid synthesis; the activity of the former supplies NADPH; the latter supplies acetyl coenzyme A. Since the catalytic capacity of malic enzyme was always increased in the presence of the A^{y} and A^{vy} alleles regardless of strain background, age or physiological conditions (WOLFF and PITOT 1972a), it may be involved in the excess fat deposition. In contrast, citrate cleavage enzyme capacity was not affected by these genes in eight-week-old mice but was greater in fourteen-week-old yellow VY males than in their black sibs (WOLFF and PITOT 1972a). Since both enzymes are required for fatty acid synthesis, it was of interest to determine whether the ratio of these two catalytic capacities might be affected by strain background or agouti locus genotype.

In inbred C3H and C57BL/6 mice this ratio was similar and much larger than in the non-yellow YS and VY mice (Table 4). In the YS and VY strains the ratio was higher in yellow than in non-yellow mice ($P = .01–.001$).

Among the F_1 hybrids, the ratio in non-yellow mice seemed to be determined by the YS and VY portions of the hybrid genomes, i.e., VY F_1 hybrids had a larger ratio than YS F_1 hybrids ($P = .01–.001$). The ratio was higher in yellow than in non-yellow mice only in the C3H × YS class ($P < .001$). Among yellow F_1 hybrids the ratio was similar in all categories except in C57BL/6 × YS mice; in this class, the ratio was smaller than in C3H × YS mice ($P = .01–.001$). These observations suggest that the *pattern* of catalytic capacities of a tissue is also under genetic regulation.

Comparison of the present data on hepatic enzymes with similar data from fourteen-week-old YS and VY strain males (WOLFF and PITOT 1972a) suggests that many of the differences in catalytic capacities found in the older mice may derive from, rather than cause, the metabolic differences between yellow and black mice. These result in a rate of weight gain between eight and fourteen weeks of age which is twice as high among yellows as among blacks.

TABLE 4

Ratio of the catalytic capacity of malic enzyme to that of citrate cleavage enzyme in livers of inbred and F_1 hybrid male mice maintained on a diet containing 7.5% fat

Strain or F_1 hybrid	Yellow ($A^{y}/–$, $A^{vy}/–$)	Non-yellow ($A/–$, a/a)
	Mean ± S.E. (N)	Mean ± S.E. (N)
C3H/HeNIcr		2.6 ± 0.3 (6)
C57BL/6JNIcr		2.5 ± 0.3 (9)
YS/ChWf	1.4 ± 0.1 (9)	1.0 ± 0.1 (9)
VY/Wf	1.4 ± 0.1 (10)	1.1 ± 0.1 (10)
C3H × YS	2.6 ± 0.2 (10)	1.6 ± 0.1 (8)
C57BL/6 × YS	1.8 ± 0.1 (10)	1.6 ± 0.1 (9)
C3H × VY	2.4 ± 0.2 (9)	1.9 ± 0.1 (10)
C57BL/6 × VY	2.3 ± 0.2 (8)	2.0 ± 0.2 (8)

The obesity and altered growth characteristics of yellow mice seem to point in the direction of hormonal factors as the prime targets of the primary effects of the A^y and A^{vy} *genes*. In 1940, WEITZE reported that parabiosis of yellow ($A^y/-$) mice with non-yellows prevented the expected obesity. These results could not be confirmed when the experiment was repeated with highly inbred YS/ChWf strain mice (WOLFF 1963). Severe deficiencies or absence of somatotrophin, thyrotrophin, and prolactin also did not prevent obesity in yellow dwarf ($A^y/a\ dw/dw$) mice (WOLFF 1965).

Recent data suggest that hormonal differences between yellow and non-yellow mice may result from, rather than be the cause of, the excess fat deposition. Serum insulin concentration and body weight of lean agouti A^{vy}/a mice resembled those of their black (a/a) sibs and were lower than these parameters in fat, mottled yellow A^{vy}/a mice of the same age (WOLFF 1971). These same data, obtained from offspring of reciprocal F_1 hybrids, also indicated that maternal effects on body weight of the yellow A^{vy}/a mice had marked parallel effects not only on the serum insulin concentration *per se* but also on its response to growth of Sarcoma 37 cells. Plasma corticosterone levels were lower in A^y/a than in a/a YS strain males, whereas there were no differences in this hormone level between yellow A^{vy}/a and black a/a mice of the VY strain (WOLFF and FLACK 1971). Responses of this hormone level to castration differed between the two strains and also between yellow and black mice within each strain. None of these parameters could be associated with differences in body weight or in the catalytic capacity of any of the enzymes assayed.

The complexity of the physiologic and biochemical data available on the "yellow mouse" syndrome suggests that the primary effect of the agouti locus mutations is exerted at a very fundamental level of cell metabolism. The lack of tissue specificity and the evidence for alteration of the tissue milieu by these mutations indicate that the agouti locus may control a ubiquitous substance, such as a cofactor, which plays an important role in determination of the intra- and extracellular environment. Future work will address itself to this possibility.

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