GROWTH OF INBRED YELLOW ($A^{y}a$) AND NON-YELLOW (aa) MICE IN PARABIOSIS[1]

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DIFFERENCES in physiological characteristics of inbred mice differing from their sibs by only a single allele provide natural experimental systems for the study of many aspects of mammalian metabolism and physiology. For the physiological geneticist such mutants are the ideal tool for the investigation of pathways by which single genes exert their "spuriously pleiotropic" effects on a wide variety of seemingly unrelated physiological and morphological characteristics. Concomitantly, such studies may also uncover hitherto unknown relationships between metabolic processes in mammals and may aid in the explanation of puzzling experimental results in various areas of biochemical and physiological investigations.

The semi-dominant lethal yellow allele (A^{y}) at the agouti coat color locus of the house mouse induces a multiplicity of physiological effects, many of which appear on the surface to be unrelated. It is therefore an excellent candidate for studies of this type. The diversity of phenotypic effects of the A^{y} allele is indicated by its lethality in homozygous form at the time of implantation, its genetic dominance over all other alleles at the agouti locus (except A^{vy}), its prevention of almost all eumelanin formation in the hair, and its induction of larger body size, increased fat deposition, increased susceptibility to lung tumor development, earlier development of mammary tumors in virgin females, and greater susceptibility to hepatoma development in males.

Sex differences in lung tumor susceptibility among inbred yellow mice (MORGAN 1950) and the obesity of most yellow mice as compared with their non-yellow sibs have led to the impression that the physiological effects of the A^{y} allele are mediated by the endocrine system. However, KASTEN (1952) was unable to demonstrate any histological differences between the thyroids, adrenals, or testes of yellow and non-yellow mice but found differences in the ovaries of yellow females as compared with non-yellow females. He concluded that the gene induced "metabolic dysfunctions involving the endocrine system" and that "obesity resulting from this hormonal disorder upsets normal ovarian function, causing sterility." WEITZE (1940) was unable to detect any histo-

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logical differences between the pituitaries of yellow and non-yellow mice.

Parabiotic union of two animals has been widely used to study the interrelationships of the endocrine glands (FINERTY 1952). This technique was used by WEITZE (1940) to detect possible hormonal differences between yellow and non-yellow mice. She found that yellow mice in parabiosis with non-yellow mice did not become obese and postulated "a hypo- or a hyperfunction of one or several of the endocrine glands" of yellow mice as the basis for this result. The rather high mortality among the parabiotic pairs used by WEITZE and the relatively undefined genetic background of her animals made a repetition of this experiment seem imperative since confirmation of her results would facilitate the determination of the specific metabolic mechanisms involved in the induction of obesity in the yellow mouse. The present investigation does not confirm WEITZE's findings. A preliminary report has appeared previously (WOLFF 1962).

MATERIALS AND METHODS

Two hundred twelve inbred mice of the YS/ChWf strain at F_{51-54} were joined in parabiotic pairs of littermates at 30 to 35 days of age. The eight experimental categories included:

		Number of pairs joined	Number of pairs surviving to end of experiment	Mortality
1. Yellow males joined with yellow males	$= A^{y}a♂ : A^{y}a♂$	16	16	0
2. Yellow male on right joined with non-yellow male on left	$= A^{y}a♂ : aa♂$	14	9	36%
3. Non-yellow male on right joined with yellow male on left	$= aa♂ : A^{y}a♂$	9	6	33%
4. Non-yellow male joined with non-yellow male	$= aa♂ : aa♂$	16	15	6%
5. Yellow female joined with yellow female	$= A^{y}a♀ : A^{y}a♀$	15	14	7%
6. Yellow female on right joined with non-yellow female on left	$= A^{y}a♀ : aa♀$	10	6	40%
7. Non-yellow female on right joined with yellow female on left	$= aa♀ : A^{y}a♀$	10	8	20%
8. Non-yellow female joined with non-yellow female	$= aa♀ : aa♀$	16	14	12%

There were no obvious differences between the data for the two heterogenic categories (A^Ya:aa, aa:A^Ya) for each sex. To compensate for the smaller numbers due to mortality in these experimental groups the data in Categories 2 and 3 (males) were combined for analysis of all aspects of this experiment. This was also done for Categories 6 and 7 (females).

Losses of individual measurements account for the variation of N for different parameters within the experimental categories in Tables 1 and 2.

Body and tail length were measured at the time of operation under sodium pentobarbital (8.57 mg/ml water) anesthesia. Body length was measured to the nearest 0.25 cm from the tip of the nose to the base of the tail while the animal was stretched to obtain the full length. Tail length, to the nearest 0.25 cm, was measured from the base of the tail to its tip. Similar measurements were obtained at the end of the experiment after sacrifice of the animals with ether.

All animals were weighed at the time of operation. Inbred littermate partners of the appropriate phenotypes were chosen whose weights were as closely similar as possible at the start of the experiments. All parabiont pairs were weighed to the nearest 0.5 gm every Friday afternoon on an Exact Weight Shadograph Scale, Model 4103-A-SA, until the end of the experiment. By weighing the mice at the same time every week, it was hoped to eliminate weight fluctuations due to the diurnal rhythm of the animals.

Old Guilford Mouse Breeder Diet (11 percent fat as corn oil) and water were fed ad libitum. Each parabiotic pair was housed separately in a 12″ × 6″ × 6″ stainless steel shoebox type cage with white pine shavings as bedding. All pairs were changed to clean cages once a week. Temperature was maintained at 76 ± 2°F and relative humidity at about 50 percent.

At 28 to 31 weeks of age the experiment was terminated. The animals were killed with ether, immediately separated, measured, and weighed. Each liver was removed and weighed on an O'Haus Centogram Balance to the nearest 0.01 gm. As soon as the liver was removed, the carcass was immersed in a glass jar filled with reagent toluene. Immediately after weighing, the liver was placed in a separate glass jar filled with toluene. The glass jars, closed with Bakelite screw caps with cork-backed tin-foil liners, were then placed in a mixture of dry ice and Dowanol (kindly provided by Dow Chemical Company). All frozen carcasses and livers were stored in a deep freeze at − 35.5°C. Toluene was chosen as the storage medium since its low specific gravity (0.87) would prevent evaporation of water from the tissues during storage, its low freezing point (− 95°C) would prevent its freezing so that the frozen carcasses and livers could be removed from the jars if necessary without previous thawing, its boiling point (110.6°C) would permit the distillation of water from the tissues, and the solubility of lipids in toluene would permit its use as fat solvent in the Soxhlet extraction without any loss of fat which might have been extracted during the storage period.

The storage jars were labeled only with the number of the parabiont pair and the number of the particular animal. In order to avoid the possibility of

unintentional bias in the results, the experimental category to which any animal belonged remained unknown until the final results were recorded.

Simultaneous determination of the total fat and water content of each liver and each carcass was performed by a method described in detail elsewhere (WOLFF and BAKAY 1963). The nonfat dry residue of each liver or carcass was calculated by subtracting its total fat and water content from its wet weight. The volume of water obtained from each liver and carcass was converted to estimated weight on a 1 ml water = 1 gm basis without correction for temperature. For purposes of this experiment such an estimate was considered sufficient.

Parabiosis method: Several modifications of the BUNSTER-MEYER method of parabiosis (1933) were found necessary. Whiskers and toe nails on the sides of the animals to be joined were clipped to prevent eye infections due to irritation, and to prevent scratching of the wound. The latter was a major problem since it prevented rapid wound healing and caused several infections and deaths. To prevent excessive stretching of the skin and consequent twisting of the parabiotic union a strip of skin about 40 to 50 mm wide was cut from the dorsal edge of one incision and from the ventral edge of the incision on the other animal. This modification decreased the mortlaity rate among the animals markedly. Two sutures of chromic gut 4–0 were placed in opposite directions through the apposed scapulae of each pair. A small incision was made in the peritoneum of each animal and the four edges were sutured together with surgical silk. The skin wounds were closed with 11 mm Michel wound clips. Approximately one week after the operation about half of the wound clips were removed. Four days later the rest of the clips were removed.

Two days after removal of the last wound clips the non-yellow partner in yellow to non-yellow pairs, or the right partner in yellow to yellow and non-yellow to non-yellow pairs, was injected intraperitoneally with 0.2 ml Evans Blue (EICHWALD, LUSTGRAAF, and STRAINER 1959). Approximately twelve hours later the uninjected partner was examined for presence of the dye to indicate the establishment of tissue connections between the two partners.

At autopsy (28 to 31 weeks of age) all parabiotic pairs were found to have extensive connective tissue connections including capillaries crossing from one partner to the other.

Statistical Analysis

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In this paper the main interest centers on the effect of three independent variables on the chosen dependent variables (body lengths, tail lengths, etc.). The three independent variables are heterogenic parabiosis versus isogenic parabiosis (p); the effect of sex, male versus female (s); and the effect of genotype, $A^{Y}a$ versus aa (g). A fourth operational factor is the choice of units of measurement that determine the level of measurement (m). Thus a measured dependent variable (y) on a particular mouse may be considered as the sum of the four effects as expressed by the equation

$$y = m + s + g + p$$

For example, for the section of Table 1 where the body lengths at four to five weeks of age are given, one can write four sets of equations as follows:

$$174m + 92s + 87g + 58p = 1405.25$$
$$92m + 92s + 47g + 30p = 754.75$$
$$87m + 47s + 87g + 29p = 703.25$$
$$58m + 30s + 29g + 58p = 463.00$$

The first equation states that the 174 mice with usable measurements had a total body length of 1405.25 cm. Among these there were 92 males, 87 yellow mice, and 58 mice which were paired in heterogenic parabiosis. The next equation expresses the characteristics of the 92 males who had a total body length of 754.75 cm. Of these, 47 males were yellow, and there were 30 males who were paired in heterogenic fashion.

The last two equations account for the characteristics of the 87 yellow mice and 58 heterogenically paired mice, respectively. By standard statistical technique, we now solve the four equations for the four unknowns.

By means of the t-test, one determines whether the factors s, g, and p are significantly different from 0. The value of m is of no biological significance. It is a purely operational factor.

RESULTS

Body and tail length: The animals to be joined were chosen according to their weight so that both partners would start out as nearly alike in weight as possible. While the body lengths of isogenic partners (A^Ya:A^Ya, aa:aa) were longer than those of heterogenic partners (A^Ya:aa, aa:A^Ya) at the beginning of the experiment (t: -2.62, D. F.: 170, $P < .01$), an inspection of Table 1 indicates that this difference occurred only among the females and is probably without significance.

At 28 to 31 weeks the heterogenic partners were somewhat longer (t: 2.54, D. F.: 164, P: .01–.02) than the animals in isogenic pairs. However, this difference is largely confined to the males.

Since the animals did not all start out at the same body length, the true measure of growth is the increase in body length between the beginning and the end of the experiment. Here we find highly significant differences among the partners in isogenic and heterogenic pairs as well as differences between yellow and non-yellow animals (Table 1). Among males and females of both genotypes, the increase in body length was consistently greater in animals joined to the opposite genotype than when joined to partners of the same genotype (t: 3.92, D. F.: 162, $P < .001$). The increase in body length was also much greater among yellow than among non-yellow mice (t: 6.04, D. F.: 162, $P < .001$). Males were longer than females, both at the beginning and the end of the experiment; however, the increase in body length was not significantly different between the sexes (t: -1.70, D. F.: 162, $P > .05$).

Tail length, surprisingly, showed a completely different pattern of growth during the experiment. At the beginning of the experiment there were no differences in tail length between animals in isogenic and heterogenic pairs, none

TABLE 1

Increase in body and tail length of yellow (A^Ya) *and non-yellow* (aa) *mice in parabiosis between 4 to 5 weeks and 28 to 31 weeks of age*

	$A^Ya♂:A^Ya♂$		$\begin{bmatrix} A^Ya♂:aa♂ \\ aa♂:A^Ya♂ \end{bmatrix}$				$aa♂:aa♂$		$A^Ya♀:A^Ya♀$		$\begin{bmatrix} A^Ya♀:aa♀ \\ aa♀:A^Ya♀ \end{bmatrix}$				$aa♀:aa♀$	
	Yellow		Yellow		Non-yellow		Non-yellow		Yellow		Yellow		Non-yellow		Non-yellow	
	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N
Body length																
4 to 5 weeks of age (cm)	8.2±.05	32	8.2±.08	15	8.2±.06	15	8.2±.05	30	8.1±.05	26	7.8±.11	14	7.7±.10	14	8.0±.07	28
Increase during parabiosis (cm)	1.7±.06	30	1.9±.09	14	1.6±.10	14	1.3±.07	29	1.7±.06	25	2.0±.14	13	1.7±.13	14	1.4±.08	27
Tail length																
4 to 5 weeks of age (cm)	6.9±.05	32	7.0±.07	15	7.0±.07	15	7.1±.07	30	7.2±.04	26	7.1±.09	14	7.0±.09	14	7.0±.06	28
Increase during parabiosis (cm)	1.3±.06	30	1.1±.06	14	1.2±.08	14	1.4±.09	29	0.9±.04	25	0.8±.06	13	0.9±.06	14	1.2±.05	27

between the two genotypes, and none between the two sexes (Table 1). However, at the end of the experiment (28 to 31 weeks of age), the tails of isogenic partners were significantly longer than those of heterogenic partners (t: -3.78, D. F.: 164, $P < .001$). Yellow mice had *shorter* tails than non-yellow mice (t: -2.73, D. F.: 164, $P < .01$), and males had longer tails than females (t: 3.34, D. F.: 164, $P < .01$). The increase in tail length was greater among animals in isogenic pairs (t: -4.53, D. F.: 162, $P < .001$), greater among non-yellow animals (t: -3.13, D.F.: 162, $P < .01$, and greater among males (t: 5.43, D.F.: 162, $P < .001$).

Greater body length of yellow mice as compared with their non-yellow littermates has been reported by CASTLE (1941) and CARPENTER and MAYER (1958). CASTLE (1941) found the tails of yellow mice to be slightly longer than those of non-yellow sibs. HESTON and VLAHAKIS (1961a, 1961b) measured total body length, including the tail, and also found yellows to be longer than non-yellows. These data were all obtained from nonparabiosed F_1 hybrid or noninbred animals so that detailed comparison with the data of the present experiment would not necessarily be meaningful. The only other reported parabiosis experiment with yellow and non-yellow mice (WEITZE 1940) did not include body and tail length measurements.

Weight gain: The weight curves shown in Figure 1 and the data in Table 2 clearly indicate that there are no effects of parabiosis on the rate of weight gain of yellow mice. This finding fails to confirm WEITZE's report (1940) that yellow mice in parabiosis with normal weight mice failed to gain weight as rapidly as yellow mice joined to each other. The actual weight gain of heterogenic (yellow:non-yellow) pairs was not different from that expected on the hypothesis that the weight gain of these pairs equals one half that of isogenic yellow:yellow pairs plus one half that of isogenic non-yellow:non-yellow pairs (Figure 1).

Carcass composition: The absence of any effect of parabiosis on the weight gain of yellow and non-yellow mice is borne out by the lack of any statistically significant effects of the heterogenic combination on the amounts or proportions of water, fat, or nonfat dry residue in the carcasses and livers (Table 2), with the exception of the weight of the nonfat dry residue of the livers. While there appear to be reciprocal physiological effects of the yellow and non-yellow phenotypes on the increase in body and tail length of both partners in heterogenic pairs, the excess fat deposition and increased amount of nonfat dry residue of yellow animals is not affected by parabiosis with non-yellow partners.

The fat and water determinations on carcasses and livers confirm WEITZE's finding (1940) that yellow mice contain more fat, more water, and more nonfat dry residue than non-yellow mice. However, water and nonfat dry residue form a smaller percentage of the total body composition of yellow mice than of their non-yellow sibs because of the greater proportion of fat in the former.

Liver composition: Livers were processed separately because of the observed high frequency of "mottled" livers which appeared to be fatty. The A^y allele apparently has similar effects on the fat and water content of liver and carcass.

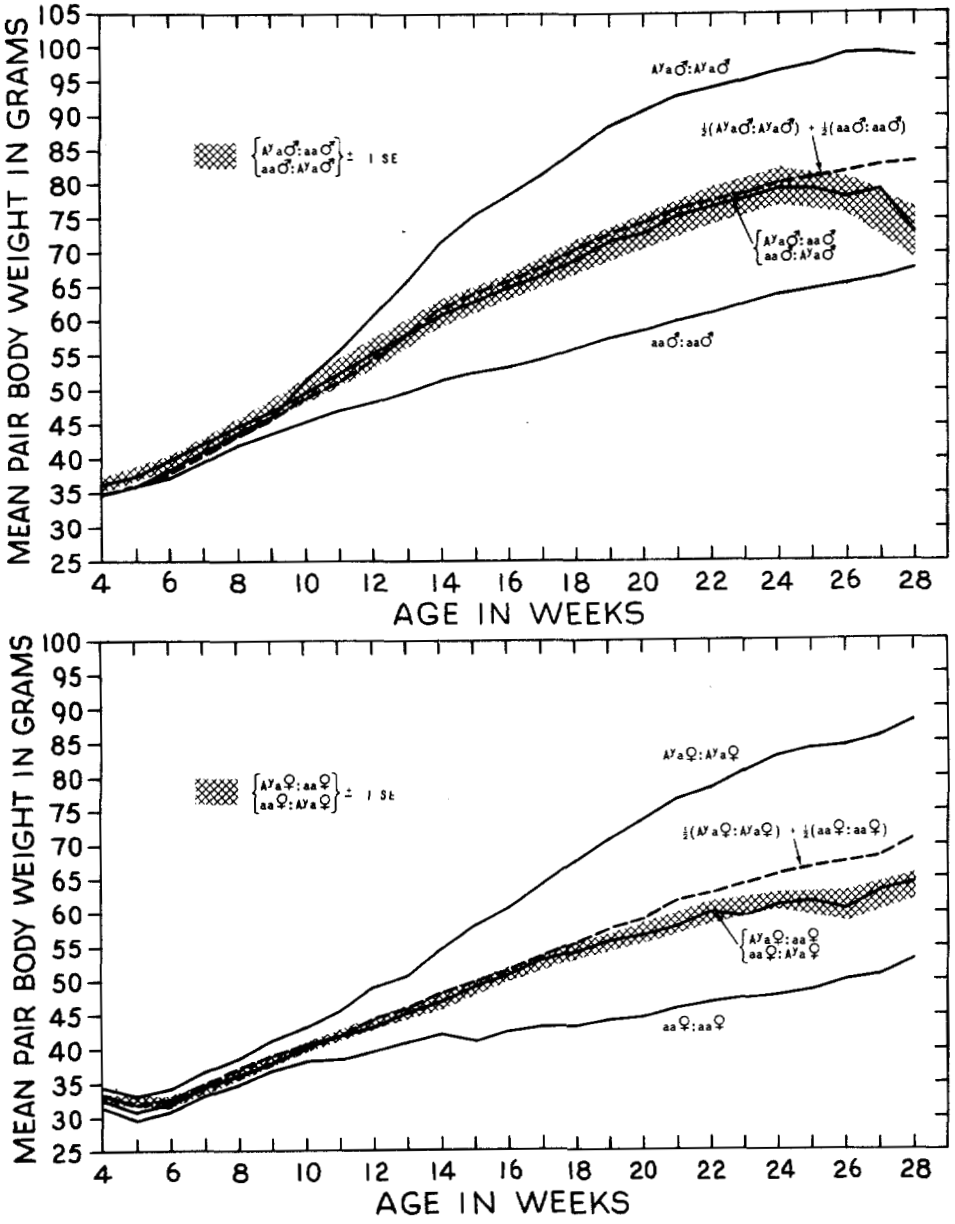

FIGURE 1.—Weight gain of inbred yellow (A^Ya) and non-yellow (aa) male and female mice in parabiosis.

However, parabiotic union of heterogenic pairs seems to decrease the amount (t: −2.66, D. F.: 167, $P < .01$), but not the proportion (t: −1.02, D. F.: 167, $P > .05$), of nonfat dry residue of the liver (Table 2). Sex appears to influence

TABLE 2

Body weight, carcass composition, and liver composition of yellow (a^Ya) *and non-yellow* (aa) *mice in parabiosis between 4 to 5 weeks and 28 to 31 weeks of age*

	$A^Ya♂:A^Ya♂$		$\left[\begin{matrix}A^Ya♂:aa♂\\aa♂:A^Ya♂\end{matrix}\right]$			$aa♂:aa♂$		$A^Ya♀:A^Ya♀$		$\left[\begin{matrix}A^Ya♀:aa♀\\aa♀:A^Ya♀\end{matrix}\right]$			$aa♀:aa♀$	
	Yellow Mean±SE	N	Yellow Mean±SE	N	Non-yellow Mean±SE	N	Non-yellow Mean±SE	N	Yellow Mean±SE	N	Yellow Mean±SE	N	Non-yellow Mean±SE	N	Non-yellow Mean±SE	N
Live body weight																
4 to 5 weeks of age (gm)	17.2±0.3	32	18.7±0.7	15	18.6±0.4	15	17.6±0.4	30	17.1±0.3	28	16.4±0.5	14	16.2±0.4	14	15.8±0.2	26
Increase during parabiosis (gm)	32.8±0.6	32	29.7±1.6	15	17.3±1.5	15	16.3±0.9	30	29.2±1.4	28	26.6±1.5	14	8.0±1.2	14	9.5±0.7	26
Carcass composition																
Wet weight (gm)	46.1±0.5	32	44.9±1.3	15	34.3±1.4	15	32.2±0.9	30	43.6±1.3	28	40.8±1.3	14	22.4±0.2	12	25.5±1.2	28
Total water (gm)	21.1±0.3	32	21.5±0.5	15	19.7±0.4	15	18.4±0.4	30	17.6±0.4	25	16.8±0.3	14	14.4±0.4	12	14.8±0.2	28
Total fat (gm)	17.2±0.3	32	15.7±0.8	15	7.2±0.9	15	7.1±0.5	30	19.7±0.9	27	18.4±0.9	14	2.7±0.5	12	4.4±0.8	27
Nonfat-dry residue (gm)	7.8±0.2	32	7.8±0.2	15	7.4±0.3	15	6.7±0.1	30	6.4±0.2	24	5.6±0.2	14	5.2±0.1	12	5.5±0.1	27
Liver composition																
Wet weight (gm)	3.9±0.1	32	3.5±0.3	14	1.6±0.1	15	1.7±0.1	30	2.7±0.2	27	2.2±0.1	14	1.3±0.1	13	1.3±0.04	26
Total water (gm)	2.2±0.1	32	2.0±0.1	14	1.0±0.04	15	1.0±0.05	30	1.6±0.1	27	1.4±0.02	14	0.8±0.1	14	0.7±0.03	26
Total fat (gm)	0.8±0.1	32	0.7±0.1	14	0.1±0.02	15	0.1±0.01	30	0.4±0.1	27	0.3±0.1	14	0.1±0.01	14	0.1±0.01	26
Nonfat dry residue (gm)	1.0±0.04	32	0.8±0.05	14	0.5±0.04	15	0.5±0.02	30	0.7±0.03	27	0.6±0.04	14	0.4±0.04	13	0.5±0.03	26

the amount (t: 5.87, D. F.: 168, $P < .001$) and proportion (t: 4.84, D. F.: 168, $P < .001$) of liver fat significantly, yellow males having higher values than yellow females (Table 2). However, yellow males have less *carcass* fat, both absolutely and proportionally, than yellow females (Table 2).

As parabiosis of $A^{y}a$ with *aa* mice decreased the amount of nonfat dry residue in the livers of both phenotypes as compared with isogenic pairs, it was decided to test whether this effect might be related to the variability of the nonfat dry residues of the carcasses. These were not affected by parabiosis of heterogenic partners (t: -0.68, D. F.: 165, $P > .05$). Covariance analysis of the nonfat dry residues of the carcasses and livers revealed that while the liver residues vary among the different experimental categories (F: 3.75, $P < .01$), this variation is independent of the variation in carcass residues (F: 2.90, $P < .01$).

Mortality pattern: A total of one hundred and six parabiotic pairs were prepared for this experiment. Out of this total, eighteen pairs died before the end of the experiment. For purposes of this discussion, any deaths occurring within one month after the operation are considered to be due to causes not related to major physiological differences between the $A^{y}a$ and *aa* phenotypes. There were only two deaths among sixty isogenic pairs more than three months after the operation—one pair of yellow females and one pair of non-yellow females. The heterogenic pairs ($A^{y}a$:*aa*, *aa*:$A^{y}a$) had a very different mortality rate; out of forty-one pairs, twelve died during this period. These were equally divided between the sexes.

All dead animals were autopsied unless autolysis was too far advanced. From the relative degrees of autolysis it appeared that in every heterogenic pair of females which died, the non-yellow partner died first. This was also the case in the two heterogenic pairs of males which were autopsied. There were no observed cases in which the yellow partner had apparently died first or almost simultaneously with the non-yellow partner. WEITZE (1940) states that the mortality among non-yellow ("A") females joined to yellow ("F") females was higher than that of the yellow partners.

In only one case, was there a marked loss of weight by the pair one to two weeks before death occurred. In this case one partner had a massive impaction and infection of the descending colon, while the other partner's spleen was greatly enlarged. In all other fatalities, if loss of weight occurred, it must have happened very suddenly—within five to six days after the previous weighing.

It is therefore concluded tentatively that the non-yellow partners died first and that the death of the yellow partner was due to the circulation of products of decomposition through the parabiotic union.

DISCUSSION

The frequently quoted report (WEITZE 1940) that parabiosis between yellow and non-yellow mice reduces the rate of weight gain as well as the amount and proportion of fat in the bodies of yellow mice has not been confirmed by the present experiment.

In the previous study, the mice used were not inbred and there is some doubt as to whether all the "F" (fat) mice actually carried the A^Y allele or were fat for other reasons. WEITZE (1940) states "All of the yellow mice became fat, whereas the grey and black animals were normal with regard to their weight. Some of the white mice became fat and others remained normal. *All the mice which show a tendency to obesity have been called F-mice* (fat mice)." While most of the F-mice used were yellow, some fat albinos were also used on the unproven assumption that the A^Y allele was present, as indicated by the obesity, although its coat color effect was not expressed because of the epistatic effect of albinism. This assumption may not always be valid since one parabiotic pair of non-yellow (*aa*) females at the end of our experiment had carcass and liver wet weights and total amounts and proportions of water and fat which were very close to the class means (Table 2) of the A^Ya:A^Ya females and not at all similar to the class means of the *aa*:*aa* females. However, in this pair of *aa* females the increase in body length (1.3 cm) was almost identical with the class mean for *aa*:*aa* females.

Differences between the diets (WEITZE used a diet containing 7.8 percent fat, ours contained 11 percent fat), other environmental conditions such as temperature, and the hereditary background of the animals may also have contributed to the divergent results. A clue to a possible major reason for this discrepancy may be provided by a comparison of the proportions of fat in single yellow and non-yellow mice, in WEITZE's yellow:non-yellow parabionts, and in our yellow:non-yellow parabionts.

Table 3 indicates that the proportions of fat in our isogenic pairs are much more similar to those found in WEITZE's single mice than to those in WEITZE's yellow:yellow and non-yellow:non-yellow pairs. This may indicate effects of the operation and the parabiotic state per se on the proportion of body fat rather than effects due to the A^Y allele. In every yellow-yellow and non-yellow:non-yellow category, WEITZE's animals had a smaller proportion of fat than the comparable single animals, while the isogenic parabionts in our experiment had about the same or a slightly higher proportion of body fat as WEITZE's single mice of the appropriate phenotype, except for the non-yellow:non-yellow female pairs. In the latter group our parabionts had 16.8 percent body fat, while WEITZE's single mice had 21.0 percent and her non-yellow:non-yellow pairs had only 8.5 percent body fat (Table 3).

In view of these apparent effects of the operation or parabiotic state on WEITZE's animals without regard to the presence or absence of the A^Y allele, the decrease in the rate of weight gain and the proportion of body fat in her yellow mice parabiosed to non-yellow mice may be related to the rather high mortality in her experiment and to the mortality pattern in our study rather than to specific effects of non-yellow mice on the rate of weight gain and proportion of body fat of their yellow partners.

WEITZE reported a 47 percent mortality (56/120) within 24 days after the operation; among the survivors there was a 47 percent mortality (30/64) by about 46 days after the operation. Therefore, the possibility that weak histo-

TABLE 3

Proportions of water, fat, and nonfat dry residue in yellow and non-yellow mice

	Percent water	Percent fat	Percent nonfat dry residue	Investigator
Yellow males				
Single	51.0	29.5	19.5	WEITZE
Parabiosed with	57.5	23.5	19.0	WEITZE
yellow male	46.6	36.0	17.6	WOLFF
Parabiosed with	62.2	13.1	24.2	WEITZE
non-yellow male	48.6	33.9	18.0	WOLFF
Yellow females				
Single	41.5	42.5	16.0	WEITZE
Parabiosed with	56.5	24.0	20.0	WEITZE
yellow female	41.5	43.4	15.3	WOLFF
Parabiosed with	70.0	5.7	24.0	WEITZE
non-yellow female	42.3	43.5	14.4	WOLFF
Non-yellow males				
Singles	63.0	13.5	23.5	WEITZE
Parabiosed with	68.5	8.2	23.2	WEITZE
non-yellow male	57.2	21.2	21.2	WOLFF
Parabiosed with	69.2	5.3	25.5	WEITZE
yellow male	57.7	20.3	22.0	WOLFF
Non-yellow females				
Single	58.5	21.0	20.5	WEITZE
Parabiosed with	67.5	8.5	24.0	WEITZE
non-yellow female	57.8	16.8	22.4	WOLFF
Parabiosed with	69.4	4.2	26.5	WEITZE
yellow female	64.1	11.8	23.6	WOLFF

incompatibility and a low degree of parabiotic intoxication (EICHWALD *et al.* 1960) may have played a role in her results cannot be excluded. The mice used were not inbred and 72 percent of the parabiotic pairs died within 46 days after the operation. WEITZE also found that the final weights of the parabionts were lower than those calculated from the average weight curves of single mice of the appropriate phenotype. She stated that the delay in growth due to the operation could not account for the total difference. In yellow:non-yellow pairs the difference was ascribed to the failure of the yellow partner to become fat. However, the divergence from the calculated weight curves of the partners in yellow:yellow and non-yellow:non-yellow pairs could not be explained.

In preliminary experiments the use of narrow parabiotic unions, similar to those used by WEITZE, beginning posterior to the shoulder and ending anterior to the hips produced a high rate of mortality within the first few weeks after the operation. Wider unions as described by BUNSTER and MEYER (1933), used in conjunction with the previously described modifications of the BUNSTER and MEYER technique, markedly reduced the mortality rate by eliminating the pos-

sibility of twisting and tearing of the union. It may be that the high rate of mortality among WEITZE's animals and in our preliminary parabiotic unions was a result of prolonged severe systemic stress (SELYE 1956), due to the almost constant twisting and pulling of the partners away from each other, with a consequent fatal exhaustion of the animals.

The mortality pattern among our parabionts may also be partly due to the gradual exhaustion of the non-yellow partners by the almost constant strain of pulling their less active and heavier yellow partners around. However, while this obvious strain is probably involved, it may be only one of several factors responsible for the mortality pattern. It is striking that the postulated exhaustion was fatal in only 14 heterogenic pairs. In the 29 surviving heterogenic pairs there was no statistically significant effect on the rate of weight gain nor on the absolute weight gain. It would be expected that non-yellow mice which were continually dragging their heavier yellow partners around would show a slower rate of weight gain and a smaller absolute weight gain than those with non-yellow partners. However, there are no significant differences in the total amount and proportion of fat, water and nonfat dry residue in the carcasses of non-yellow mice between heterogenic and isogenic pairs (Table 2).

As the nonfat dry residue (chiefly protein and glycogen) of the livers of yellow males and females is decreased by heterogenic parabiosis without a concomitant decrease in the nonfat dry residue of the carcass, it might be postulated that metabolic changes in the liver induced by the interaction of the yellow phenotype with the non-yellow phenotype are involved in the mortality pattern.

Since the A^{y} allele, maleness, and heterogenic parabiotic partners, all exert statistically significant effects on the amount of nonfat dry liver residue, it appears that the liver is affected by the postulated altered metabolic and hormonal balance of the mouse. This is also indicated by the higher incidence and larger number of hepatomas which develop in yellow ($A^{y}A$ (C3H $\times$ YBR)F_1 males as compared to their non-yellow (AA) brothers (HESTON and VLAHAKIS 1961a). ZOMZELY and MAYER (1959) reported a higher rate of incorporation of labeled acetate into liver cholesterol in yellow as compared with non-yellow mice, while the rates of incorporation into carcass cholesterol showed no such differences bewteen the two phenotypes. They also found the cholesterol content of livers of yellow mice to be higher than that of non-yellow mouse livers; the percentage of total cholesterol synthesis contributed by the liver was two to three times larger in yellow mice than in non-yellows. When these findings are considered in conjunction with the effect of heterogenic parabiosis on the nonfat dry residue of the liver, with no such effect on the carcass, it seems that the possibly altered metabolic balance in the yellow mouse may affect liver metabolism to a somewhat greater extent than metabolic processes in other tissues.

Physiological differences between animals elicited by parabiosis are usually interpreted as being hormonal in nature (FINERTY 1952). While proof is lacking in the present case that the reciprocal effects on body and tail growth of the two phenotypes are hormonal, it would seem indicated that this possibility should be explored.

It is probable that increased body length and tail length are partly due to increased growth of the vertebrae. In this case two pituitary hormones known to affect bone growth, namely somatotropin and thyrotropin, may be involved in the observed results. Since effects of parabiosis of yellow to non-yellow mice on body length increase are the reverse of those obtained on tail length increase, and since *both* yellow and non-yellow partners are affected in the same direction in each case, it is obvious that the interactions of the two different physiological systems must be quite complex and reciprocal. A clue may lie in the fact that in $A^Ya:A^Ya$ pairs the body length is greater and the tail length smaller than in *aa*:*aa* pairs. Non-yellow animals in heterogenic pairs show a greater increase in body length and a smaller increase in tail length than non-yellow animals in isogenic pairs. Thus, it appears that yellow partners influence the body and tail growth of their non-yellow partners. However, yellow partners in heterogenic pairs *also* show a greater increase in body length and a smaller increase in tail length than yellow animals in $A^Ya:A^Ya$ pairs. It is thus apparent that there is a type of reinforcement of the effects of the yellow phenotype by the non-yellow phenotype.

Such changes in the allometric growth pattern of the body and tail as elicited here by parabiosis partly parallel the larger body size and smaller tail length of homoiothermic animals living in cold climates as compared to related species living in warmer climates. The latter observations have been interpreted as adaptations to body temperature regulation (BERGMANN 1847; ALLEN 1905).

Experimentally these observations have been confirmed by OGLE (1934). In an 88 to 92°F environment male "white mice" of a noninbred stock had short bodies and long tails, while in a 64°F environment the bodies were of the same length as the controls (70 to 80°F) although the tails were 1 cm shorter than those of the controls.

HARRISON, MORTON, and WEINER (1959) found that a 90°F environment increases the rate of tail growth and that the relative increase in the tail-growth rate is greater in those inbred strains which have smaller control tail lengths.

If these environmental temperature effects on body and tail length are at least partly related to heat exchange regulation, it becomes possible to speculate on possible hormonal factors involved in our results.

In our inbred YS/ChWf strain essentially all yellow animals become heavier than their non-yellow littermates. However, this condition is apparently not true in all strains carrying the A^Y allele (FENTON and CHASE 1951). TURNER (1948) reports that obese yellow mice had a lower rectal temperature and were less able to adjust to low environmental temperatures (5°C and 10°C) than nonobese yellow mice. RYTAND (personal communications cited by TURNER 1948) found no significant differences in oxygen consumption of obese and thin yellow mice at room temperature. However, the Q_{O_2} (ml O_2/g/hr) of these obese yellow mice ($3.45 \pm .008$) was significantly lower ($P < .0001$) than the Q_{O_2} of nonobese yellow mice ($5.29 \pm .22$) according to TURNER (1948).

These reports suggest that thyrotropin and/or thyroxine may be hormonal factors affected or mimicked by the A^Y allele. Thyroxine has been suggested by

other investigators as possibly being involved in the "yellow mouse syndrome."

A study of the effects of growth hormone and thyroxine on tail length increase in rats suggests that the results obtained in our experiment may be due, at least in part, to different effective levels of these two hormones. VAN DYKE, SIMPSON, LI and EVANS (1950) found that 5μg thyroxine injected with 200μg somatotropin (STH) gave a 75 percent greater increase in tail length in hypophysectomized rats than that obtained with 200μg STH alone. This synergistic action of thyroxine with growth hormone on tail length increase may partly explain the smaller increase in tail length of the yellow mice if a lower *effective* level of thyroxine in these animals is postulated. However, the greater increase in body length of the same yellow mice indicates that such a hypothesis is probably too simplified and that variations in the effective levels of, at least, thyroxine and growth hormone may be involved in this particular effect of the A^Y allele.

There are several possible ways, which are not necessarily mutually exclusive, in which the A^Y allele could affect the apparent levels of growth hormone and thyroxine. The A^Ya phenotype could increase synthesis and release of growth hormone and decrease the synthesis and release of thyroxine by direct or indirect effects on the pituitary and thyroid glands. The rates of catabolism of these hormones could be changed by the A^Ya phenotype so that their plasma concentrations would be maintained at different levels than in the *aa* phenotype. The sensitivity of the target cells to these hormones could be altered by the A^Ya phenotype so that equal plasma concentrations of the hormones would produce greater or smaller responses than in the *aa* phenotype. Mimicry of the cellular effects of growth hormone, thyrotropin, and thyroxine by the A^Ya phenotype is another possibility. In this case the A^Y allele would alter cellular metabolism so as to achieve physiological effects similar to those which increased or decreased levels of various hormones would induce in *aa* mice.

The last possibility is attractive as a working hypothesis because it might eventually lead to an explanation of not only the seemingly "hormonal" effects of the A^Ya phenotype but also of metabolic effects which are not obviously hormonal. Among the latter would be included the lethality of the A^YA^Y embryos at the time of implantation (ROBERTSON 1942, EATON and GREEN 1963), the lowered requirement for sulfhydryl compounds by A^Y- melanocytes, *in vitro*, for the production of phaeomelanin (CLEFFMANN 1963), and the modification of the hair bulb environment of melanocytes by the A^Y- phenotype (SILVERS 1961).

SUMMARY

Increase in body and tail length and the rate of body-weight gain between 4 to 5 weeks of age and 28 to 31 weeks of age were measured on 89 parabiotic pairs of yellow (A^Ya) and non-yellow (aa) mice of the inbred YS/ChWf strain. At the end of the experiment, total fat, water, and nonfat dry residue in the carcasses and livers of each member of the like-sexed yellow to yellow, yellow to non-yellow, and non-yellow to non-yellow pairs were determined.

In heterogenic (yellow to non-yellow) pairs the body length increase of both partners was enhanced in comparison with isogenic pairs, while the tail length increased less in both partners of heterogenic pairs than in comparable isogenic pairs.

Parabiotic union of inbred yellow with non-yellow mice had no effect on the rate of weight gain of either partner.

Increase in body weight between 4 to 5 weeks of age and 28 to 31 weeks of age was greater in yellow mice than in non-yellows and greater in males than in females. Yellow males and yellow females weighed almost the same at the end of the experiment, while non-yellow males were much heavier than non-yellow females.

Analysis of the composition of the carcasses showed statistically significant greater wet weight, water content, fat content, and amount of nonfat dry residue in yellow mice than in non-yellows. The proportion of fat was higher in yellow mice, but the proportion of water and nonfat dry residue was lower than in non-yellow mice. Males had a higher wet weight, more water, and more nonfat dry residue than females but the same fat content. There were no effects of parabiotic union with a heterogenic partner on carcass composition.

Liver composition was affected by the A^Y allele in a similar manner as carcass composition. Male livers had a higher wet weight, more water, more fat, and more nonfat dry residue than females. Males had a higher proportion of fat in the liver than females. This was especially true among yellow mice. The amount of nonfat dry residue of the liver was significantly decreased in both partners of yellow:non-yellow pairs as compared with isogenic pairs.

Among sixty-four *isogenic* pairs, only two died three months or more after the operation. However, out of forty-one *heterogenic* pairs twelve died during the same period.

It appears that there are reciprocal physiological effects, possibly hormonal in nature, of the parabiotic partners on each other which are induced by the A^Ya phenotype.

If all reported physiological characteristics of yellow mice are considered as being phenotypic manifestations of a single primary action of the A^Y gene, and in view of the failure of several investigators to detect histological abnormalities in various endocrine glands, it is proposed, as a working hypothesis, that the effects of this allele on cellular metabolism may mimic metabolic effects due to changes in the relative *effective* levels of several hormones in the extracellular fluid.

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