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An inherited mild middle-aged adiposity in wild mice

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SUMMARY

In a warfarin-resistant population of wild mice reared in the laboratory, a dominant gene for adiposity, *Ad*, was found to segregate. The onset of obesity is at 4–6 months, and adipose mice suffer from hyperinsulinaemia; the sexes differ in penetrance, males having greater penetrance then females. Linkage backcrosses show the gene to be situated on chromosome 7 with about 25% recombination with the closely linked warfarin-resistance genes *War*, and frizzy, *fr*. The finding of adipose in two other wild populations also carrying *War* is discussed as an ecological and physiological problem.

INTRODUCTION

The well-known mouse genes for obesity and diabetes, *ob* and *db*, have provided useful models for the study of similar human conditions (Coleman, 1973; Coleman & Hummel, 1973). However, they are both somewhat unlike the commoner human forms of these conditions in that the obesity is gross, onset is early in both mouse genes, and the diabetes is characterized by both hyperinsulinaemia and glycosuria; also both are recessive, *obob* is sterile, and so they pose certain maintenance problems.

This paper concerns a new mouse gene, adipose, which is dominant. It has been given the symbol *Ad*, and is not to be confused with the symbol *ad* given to a recessive adipose gene now known to be identical with *db*: the symbol *ad* has been withdrawn and the *ad* mouse is now referred to as db^{ad} (Falconer, 1973).

In *Ad*+ and *AdAd*, adiposity is developed in middle age and does not seriously reduce activity or fertility; it is characterized by hyperinsulinaemia, but glycosuria is not consistently present. It has the added interest that it has been detected only in laboratory descendants of wild mice: its function in the three wild populations in which it must have segregated is not clear, and it may in fact express differently (i.e. not as adiposity), or not at all, in the wild environment where activity is unrestricted and food shortages are common (Trayhurn, James & Gurr, 1979).

MATERIALS AND METHODS

In 1971 a colony of wild mice was set up from six individuals carrying the *C* and c^e alleles (wild-type colour and extreme chinchilla, chromosome 7), in order to study whether the spread of the c^e allele, as judged from trappings in Cambridge

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during warfarin-baiting, was due to linkage with a major warfarin-resistance gene. This in fact was found to be the case, the gene, symbolized *War*, being located very close to the frizzy, *fr*, locus; it is uncertain whether it is on the colour side of frizzy or on the other side. *War* is thus in the same position relative to the colour and pink-eyed dilution gene, *p*, as is the warfarin-resistance gene *Rw* in the analogous chromosome of the rat (Wallace & MacSwiney, 1976). The mouse resistance gene is almost fully penetrant in females and has low penetrance in males.

Mice were obtained from wild resistant populations in two other places in England, Loughborough and Nottingham, and a similar study made: the *War* gene was proved to exist in both populations, and, besides the sex difference in penetrance, differences in penetrance due to genome were found (MacSwiney & Wallace, 1978).

During the early stages of the above work, the Cambridge colony, known as *PBI*, was divided into two subcolonies, one *CC* and one $c^e c^e$. Both were selected for resistance during 2 generations, and then mice from the *CC* colony were outcrossed to a susceptible chromosome 7 marker strain (homozygous for *fr*, shaker-1, *sh*-1, chinchilla, c^{ch} and *p*), and backcrosses were made to the susceptible stock. The above mapping was based on the backcross segregations.

Expression of the adipose gene

During the subdivision of the colony, several mice with a rotund outline were observed. This 'chubby' condition was seldom seen before 4 months old and was well developed by 6 months: the transition in individual mice took about one month. Matings were made: chubby by chubby, chubby by normal (reciprocally for sex), and normal by normal. For the matings, animals exceeding 28 g were chosen as chubby mates, but for their progeny, 'chubbiness' was judged by eye. The results are given in Table 1. (A shortage of males is due to fighting on storage so that some had to be killed.)

It was suspected that there were three phenotypes in the progeny in Table 1, and when the outcrosses in the linkage study (see below) revealed the condition to be dominant, they were classified as $++$, $Ad+$ and $AdAd$. To discover their frequencies, and to study their relation to warfarin-resistance, a representative sample of these progeny (119 animals) were subjected to the standard resistance test for 3 weeks, starting at 4 months of age or older, and observed for a further month (see Wallace & MacSwiney, 1976). The date of onset of adiposity was noted: this was in all cases clear before the end of the test or the ensuing month. The results are given in Table 2. (The table lacks female susceptibles because the stock was *War War* and the *War* gene is fully penetrant in females only.)

To discern the relations between fat deposition, weight and food intake, these 119 mice were weighed during the test (daily for half of them but weekly for the rest as this was found sufficient), and their daily intake of warfarin bait was also recorded (by noting the loss from the supply – which was always in excess of need). They were autopsied at the end of the month. (Data for the six which did not survive are not available as the haemorrhaging due to susceptibility obscured

the fat deposits; also there was always about 3 days of extreme weight loss and lowered intake preceding death.) The surviving 66 females and 47 males were, on autopsy, scored for fat deposit according to a 5-point scale:

0 = no extra fat,
1 = little extra fat, usually renal,
2 = extra fat, usually renal and lower abdomen,
3 = as for 2 but fat more extensive,
4 = as for 3 but larger amounts and occurring also subcutaneously.

The relation between the adipose phenotypes (i.e. 'chubby' status) while alive, and relation to fat deposit and weight at autopsy, is given in Table 3.

Data on weight and intake accrued also from the linkage studies (see next section). These were added to the above study, and the results from all the mice so investigated are given in Table 4.

The susceptible male mice in the *PBI* linkage study are pooled into two classes: normal and adipose. The number of days these took to die during the warfarin baiting is given in Table 5.

Linkage studies

In the first *PBI* backcross study on the *War* gene, adiposity was observed in the F_1 of the outcrosses, thus proving the condition to be dominant, and it segregated in the backcross progenies, proving itself to be linked with frizzy (Wallace & MacSwiney, 1975). Details of this and the Nottingham and Loughborough studies, in relation to adiposity, are now given.

In the Cambridge *PBI CC* study, 9 *War War* mice were used in the outcross to the susceptible marker stock: four females were adipose and none normal, three males were adipose and two normal. From each outcross, 5–6 F_1 were used: more than half the 47 F_1 were adipose, proving, on the supposition of one main locus for adiposity that all the outcross animals, whether phenotypically adipose or not were *AdAd*. In the total F_1, 39 females and 8 males were backcrossed each to one normal susceptible marker strain animal. Adiposity in the F_1 developed at an average age of 3 months, and the distinction between normal and adipose was clear at 4 months.

The backcross generation consisted of about ten mice from each F_1 (from the females' first two litters); they were chosen so that each F_1 contributed frizzy and normal in roughly equal numbers. They were reared to 4 months and subjected to the standard 3-week warfarin test. They were weighed at the beginning of the test and at least weekly thereafter, and their daily food intake was assessed throughout the test by comparing the amount available on one day at 9 a.m. (always in excess of need) and the amount left on the next day at 9 a.m. Those that died before the end of the test were classified as susceptible and their average weight and daily intake noted until death. Those that lived until a month after the test were classified as resistant and their average weight and daily intake noted. For all the mice, their normal or adipose status was assessed during the test, judging by the body outline, and at death or at the end of the test by autopsy,

Table 1. *Matings within the Cambridge PBI mice with parental and offspring chubby status*

Female parent ...	Chubby ×		Chubby ×		Normal ×		Normal ×			
Male parent ...	chubby		normal		chubby		normal		Totals	
	F	M	F	M	F	M	F	M	F	M
Normal offspring	4	0	36	18	15	27	107	35	162	80
Chubby offspring	50	44	22	19	48	29	21	46	141	138
Totals	54	44	58	37	63	56	128	81	521	
Contingency χ^2	$>0{\cdot}50$		$1{\cdot}29$		$6{\cdot}71$		$35{\cdot}30$		$14{\cdot}34$	
Probability	$>0{\cdot}30$		$>0{\cdot}20$		$<0{\cdot}01$		$\ll 0{\cdot}001$		$<0{\cdot}001$	

judging by their fat deposits: the two assessments coincided in the majority of cases. The data were collated and summarized in the form published for the warfarin study (Wallace & MacSwiney, 1976, p. 177) but subdivided into the two further classes adipose and normal. It was checked that the adipose:normal observations from the phenotypically *Ad* F_1 agreed with those from the phenotypically impenetrant *Ad* F_1, and that these observations also agreed as between female and male heterozygous F_1. It was also checked that the *Ad*:+ observations were nearly independent of the *C* and c^{ch} classes, the normal and shaker-1 classes, and the normal and *p* classes: that is, adiposity is loosely linked with, or independent of, these alleles and does not interact with them.

In the Loughborough and Nottingham studies the outcross animals (one Loughborough and two Nottingham) were not adipose and it was not possible, because of the small numbers involved, to deduce the *Ad*+ status of the F_1; but the segregation of adipose and normal mice in the backcross data in roughly the same proportions within sexes as in the Cambridge data, and with the same relation to colour, shaker and pink-eye markers, showed that their backcross progeny were genetically fully comparable. These mice were warfarin-tested and their adipose status judged, in exactly the same way as in the Cambridge *PBI* backcross mice. The results of all three studies are given in Table 6.

RESULTS

Expression of Adipose gene

It is clear in Table 1 that the more 'chubby' appears in the parents, the more it appears also in the offspring, i.e. this feature is inherited. It might be thought to be infectious if it were not for the fact that the normal mice never developed the 'chubby' feature of their mates. There is also a marked overall tendency for female progeny to be normal compared with male, a sex difference not reflected in the 'chubbies'. This discrepancy is consistent with a sex-limited expression.

If there is a single gene, it should have the same frequency in both sexes. It is clear from Table 2 that although the frequency of the *AdAd* phenotype is closely similar in the sexes (about 23%), that of the *Ad*+ and + + is markedly different. This suggests full, or at least equal penetrance of *Ad* in the homozygote

Table 2. *Offspring of matings in Table* 1, *classified according to warfarin-resistance and adipose status*

	Females				Males			
	++	*Ad*+	*AdAd*	Total	++	*Ad*+	*AdAd*	Total
Resistant ...	34	15	17	66	8	29	10	47
Susceptible ...	0	0	0	0	1	4	1	6

Table 3. *Relation of weight to fat deposit in adipose phenotypes*

	++	*Ad*+	*AdAd*
	Females		
Fat score	0, 1, 2	1, 2, 3	3, 4
Weight (g)	Most < 20 Few slightly > 20	Most > 20 Few slightly > 25	All > 25
	Males		
Fat score	0, 1, 2	2, 3	3, 4
Weight (g)	Most < 25 Few slightly > 25	25 to 32 Few 32–34	Most > 32

in the two sexes, but misclassification, at least in females, of *Ad*+ as ++. The data for males, although small, indicates no gross relationship between *Ad* and *War*.

Table 3 shows that, although 'chubbyness' as a criterion of the adipose phenotypes, is broadly reflected in fat deposition score and weight, there is some overlap in both types of measurement. The 'by eye' classification (i.e. chubby status) seems to give three phenotypes more distinctly than does either of these more objective measurements taken separately. This is possibly because the *PBI* population segregates in polygenes affecting these measurements, in addition to the single major gene for adipose.

Table 4 shows that there is reasonable agreement in the weights and in the intakes in the three sources of data, so that conclusions may be drawn from the averages based on all the data pooled. Mean weights between the three *Ad* phenotypes in each sex are different; however, the standard errors preclude significance at the 5% level. This is true also of the intakes. However, the lower weights have the lower intakes, so that there is an overall correlation between weight and intake. The lower part of this table shows a weak correlation between weight and intake within phenotypes. It is noticeable that the *AdAd* are very much heavier than ++ but without as great an increase in intake: it therefore seems possible that they do not get fat because they eat more, but that the physiological defect is in a low level of energy expenditure which results in a high efficiency. Separate tests of the effect of unrestricted activity and of unrestricted diet would throw more light on the physiology of the defect.

An observation worth noting here is that the onset of adipose in the F_1 of the linkage study was at 3 months, i.e. earlier than in the *PBI* stock. This shows that genetic milieu affects expression.

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Table 4. *Weight (Wt) and food intake (In) means (g) for the total (T) survivors of warfarin tests*

Phenotype ...	++			*Ad+*			*AdAd*		
	Wt	In	T	Wt	In	T	Wt	In	T
				Females					
Linkage backcross	20·82	3·42	103	23·99	3·72	15	—	—	—
F_1 of linkage outcross	18·35	3·09	31	22·22	3·57	7	—	—	—
Pure PBI mice	18·10	3·18	34	24·12	3·33	15	36·47	3·82	17
Overall mean	19·82	3·31	168	23·74	3·53	15	36·47	3·82	17
S.E.	2·45	0·43	—	2·87	0·41	—	4·44	0·30	—
Coeff. of correlation, r	0·2236			0·2236			0·1825		
Coeff. of determination, r^2	0·05			0·05			0·03		
				Males					
Linkage backcross	23·75	3·35	19	26·51	3·58	24	—	—	—
F_1 of linkage outcross	22·94	3·25	6	31·11	3·69	2	—	—	—
Pure PBI mice	20·24	2·92	8	25·85	3·27	29	35·33	4·06	10
Overall mean	22·75	3·23	33	26·37	3·42	55	35·33	4·06	10
S.E.	2·16	0·49	—	3·46	0·47	—	2·69	0·28	—
Coeff. of correlation, r	0·4006			0·6599			0·2149		
Coeff. of determination, r^2	0·16			0·43			0·05		

Table 5. *Number of days to death of susceptible normal and adipose males (linkage backcross data)*

Days to death ...	0–3	4–6	7–9	10–12	13–15	16–18	19–21	22	Total
No. of males									
normals	9	47	27	6	3	2	2	18	114
adipose	1	24	42	11	4	0	1	24	107

Table 5 shows that the modal survival period for *Ad+* is about 3 days longer than that for normal mice. The daily weight records show that this is due to the fact that, after both kinds of mice stop eating the bait, the adipose ones lose weight about 3 days later than the normal ones: it may thus be simply that the fatty deposits are drawn upon for the energy normally supplied by food, until they are at a low ebb.

Other aspects of the physiology of adipose (homozygotes) are: the extra fat is accommodated in males by an increase in fat cell size with no change in cell number. In females, however, there is an increase in both the fat cell size and number. The adipose animals are characterized by hyperinsulinaemia but there is no hyperglycaemia. These features are described fully elsewhere (Trayhurn *et al.* 1978).

Table 6. *Segregation of progeny at 4 months from* Ad War/fr sh-1 $c^{ch}p$ *of both sexes mated to multiply recessive*

Wild origin of *Ad* and *War* genes		Females: War +	+ fr	War fr	+ +	Total	Males: War +	+ fr	War fr	+ +	Total
PBI colony	Ad	15	3	0	3	21	24	22	0	57	103
Cambridge	+	101	90	2	14	207	19	68	0	29	116
		116	93	2	17	228	43	90	0	86	219
Loughborough	Ad	2	0	0	0	2	9	4	0	1	14
	+	13	16	0	0	29	5	13	0	0	18
		15	16	0	0	31	14	17	0	1	32
Nottingham	Ad	5	1	0	0	6	9	2	1	1	13
	+	9	15	0	2	26	7	18	0	3	28
		14	16	0	2	32	16	20	1	4	41
Grand total	Ad	22	4	0	3	29	42	28	1	59	130
	+	123	121	2	16	262	31	99	0	32	162
		145	125	2	19	291	73	127	1	91	292
Estimated recombination values on combined data:		*War-fr* $1{\cdot}75 \pm 0{\cdot}01\,\%$					$1{\cdot}73 \pm 0{\cdot}03\,\%$				
		Ad-fr $17{\cdot}12 \pm 0{\cdot}51\,\%$					$26{\cdot}89 \pm 0{\cdot}11\,\%$				
		Ad-War $24{\cdot}91 \pm 6{\cdot}92\,\%$					$34{\cdot}74 \pm 5{\cdot}67\,\%$				

Linkage studies

The backcross progeny for the Cambridge, Loughborough and Nottingham mice are set out in Table 6. The *Ad*:normal observations are homogeneous within sexes ($P > 0{\cdot}10$). The *War*:normal observations are homogeneous within the females ($P > 0{\cdot}80$) but heterogeneous within males ($P < 0{\cdot}01$), as expected from the analysis already published (MacSwiney & Wallace, 1978); however, the figures for the aberrant population (Nottingham) are small, and so are pooled for the grand totals. It can also be seen, by comparing the 2×2 observations within the *War-fr* complementary pairs, between colonies, that they agree in the relation between *Ad* and *fr* and in the relation between *Ad* and *War*. (For example, the first left 2×2 in the Cambridge data, namely $\begin{smallmatrix}15 & 3\\ 101 & 90\end{smallmatrix}$ agrees with its counterpart in *the Loughborough and in the Nottingham data.) The grand totals may therefore* be used for the interpretation of the *Ad-War-fr* relationships.

The recombination values and standard errors (Bailey, 1961) prove conclusively that *Ad* is linked with *War* and *fr* and the following order is indicated:

Ad–War–fr.

It is interesting to note that the large standard errors for the *Ad-War* linkage almost allow for an independence interpretation, although the other smaller standard errors preclude it. This is due to the fact that in the *Ad/War* data *Ad* penetrates mainly in males and *War* mainly in females. (Impenetrance percent for *Ad* is 81·61 for females and 15·73 for males, while that of *War* is 10·01 for females and 54·71 for males.) It is as if each gene were segregating reliably in two different bodies of data, the female and the male. Unless this unusual situation

Table 7. *Observations on wild mice in natural environment and on breeding many generations in the laboratory*

	Natural environment				Laboratory cages	
	Warfarin baited	Predation	Cold season	Food shortages	*War* gene segregated	*Ad* gene segregated
Israeli	Unknown	Probably	Yes	Unlikely	Unknown	No
San Francisan (coalmine)	No	No	No	Unlikely	Unknown	No
Skokholm Island	No	No	Yes	Yes	No	No
Peru (agric. area near Lima)	Sporadic or none	Yes	Yes	Unlikely	Unknown	No
PBI Cambridge	Heavily	Some	Yes	Yes	Yes	Yes
Loughborough	Heavily	Some	Yes	Mild	Yes	Yes
Nottingham	Heavily	Unknown	Yes	Mild	Yes	Yes

is understood it might be thought that *Ad* is not very certainly linked with *War* while being firmly linked with its very close neighbour *fr*.

DISCUSSION

It is remarkable to find a gene like adipose in wild populations. It would appear to have several disadvantages: hyperinsulinaemia and insulin resistance, possibly reduced agility, and, if it is like the *ob* and *db* genes, a reduction in survival in cold conditions. And it also has an advantage in that a fat animal can survive food-restriction longer.

It is more remarkable that it is found only in the three wild populations known to be warfarin-resistant. Table 7 shows, for the wild populations studied by the first author (M.E.W.), what is known about their natural environments and their genetics as found in laboratory cages. The most consistent feature is the association of adipose with warfarin-baiting. This suggests that adipose has some advantage only in such an environment, and that this advantage outweighs the disadvantages. The observation that death is delayed by about 3 days on an exclusively warfarin diet in the laboratory, suggests that in natural conditions where there is other food about (though it may be scarce) death may be delayed longer – and even be avoided during short periods of semi-starvation. How this is mediated can only be guessed: perhaps warfarin, which dissolves in fat, is absorbed straight into the fat of adipose animals, thereby making the nutritious part of the bait available for food and energy (P. Trayhurn, personal communication).

On the other hand, adipose may not express itself in the wild in any of the disadvantageous ways found in laboratory conditions. If it is not fat and there is high efficiency, these features would be advantageous. In this case, it would be a good model for the common mild middle-aged obesities in man, who also may express them only in the artifical civilized environment of affluence and exercise restriction (James & Trayhurn, 1976). The study of a wild population containing both the *Ad* and *War* genes in which these genes' frequencies are known, would throw light on the many questions posed by our findings.

REFERENCES

BAILEY, N. T. J. (1961). *Introduction to the Mathematical Theory of Genetic Linkage.* Oxford: Clarendon Press.

COLEMAN, D. L. (1973). Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia* **9**, 294–8.

COLEMAN, D. L. & HUMMEL K. P. (1973). The influence of genetic background on the expression of the obese (ob) gene in the mouse. *Diabetologia* **9**, 287–93.

FALCONER, D. S. (1973). Allelism of adipose (ad) and diabetes (db). *Mouse News Letter* **48**, 27.

JAMES, W. P. T. & TRAYHURN, P. (1976). An integrated view of the metabolic and genetic basis for obesity. *Lancet* ii, 770–73.

MACSWINEY, F. J. & WALLACE, M. E. (1978). Genetics of warfarin-resistance in house mice of three separate localities. *Journal of Hygiene* **80**, 69–75.

TRAYHURN, P., HAWKINS, R. A., JAMES, W. P. T., GURR, M., MACSWINEY, F. J. & WALLACE, M. E. (1978). Ad – a new obese mutant. *International Journal of Obesity* **2**, 75.

TRAYHURN, P., JAMES, W. P. T. & GURR, M. I. (1979). Studies on the body composition, fat distribution and fat cell size and number, of *Ad*, a new obese mutant mouse. *British Journal of Nutrition* **41** (in the press).

WALLACE, M. E. & MACSWINEY, F. J. (1975). Warfarin resistance and a new gene for obesity. *Mouse News Letter* **53**, 20.

WALLACE, M. E. & MACSWINEY, F. J. (1976). A major gene controlling warfarin-resistance in the house mouse. *Journal of Hygiene* **76**, 173–81.