Studies on the body composition, fat distribution and fat cell size and number of '*Ad*', a new obese mutant mouse

BY P. TRAYHURN AND W. P. T. JAMES

Dunn Nutrition Unit, University of Cambridge and Medical Research Council, Milton Road, Cambridge CB4 1XJ

AND M. I. GURR

Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ

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1. Studies have been performed on the body composition, the fat distribution, the fat cell size, and the 'observable' fat cell number of a new obese mutant, the Adipose (*Ad*) mouse. The serum glucose and insulin concentrations have also been investigated. All studies were undertaken with animals aged 6 months.

2. The obese animals weighed over 50% more than the lean, but there was no difference in the body or tail lengths.

3. The obese animals had an increase in the weight of the liver, but the increase was only proportional to the increase in the total body-weight.

4. The carcasses of the obese mice contained more water as well as more fat than those of the lean. In the males the fat content was 3·9 times greater, while in the females it was increased by 5·5 times.

5. The nitrogen content of the defatted dry carcass was the same in both lean and obese animals but the total body protein was higher in the obese.

6. Fat was dissected from four major depots, gonadal, abdominal, hind subcutaneous and interscapular subcutaneous (including brown adipose tissue), and each was substantially larger in the obese animals. This indicated that the additional fat of the *Ad* mouse was not localized to any particular site.

7. In *Ad* males there was no over-all increase in the observed number of adipocytes, all the extra fat being accommodated by an increase in fat cell size (3·8 times). However, in *Ad* females there was a 3·3-fold increase in the number of observable fat cells as well as a 2·2-fold increase in fat cell size.

8. Non-fasted obese animals were not hyperglycaemic, but there was a 5·3-fold increase in the concentration of serum insulin. Hyperinsulinemia in the presence of normoglycaemia suggested that the obese animals were insulin resistant.

Obesity in animals has been extensively studied in attempts to gain insight into the causes of human obesity. Animal obesity can be either genetically transmitted or experimentally induced. In the latter, obesity is produced by manipulations such as, hypothalamic lesioning, the administration of insulin, or by feeding a high-fat diet (Bray & York, 1971). The genetic forms of obesity have been classified as being either single gene, inbred, hybrid, or polygenic (Staats, 1964). The single-gene group can be further divided into those strains where the inheritance is recessive, and those where it is dominant. Many of the mutants where obesity is recessively inherited, the obese-hyperglycaemic (*ob/ob*) mouse, the diabetic (*db/db*) mouse and the fatty or Zucker (*fa/fa*) rat, are widely used in obesity research.

Until recently the yellow obese mouse (A^y, A^{vy}), first described by Cuenot (1905), was the only known dominantly inherited obese mutant. However, Wallace & MacSwiney (1975) reported the presence of a new mutation in a group of wild mice caught in 1971 in the grounds of the Plant Breeding Institute, Cambridge. The new mutant was given the gene symbol '*Ad*'. The recessively inherited mutant which formerly held this name (*ad/ad*) is now considered to be allelic with the diabetic (*db/db*) mouse and is referred to as db^{ad}/db^{ad} (see Herberg & Coleman, 1977). The *Ad* mutation in the mild genetic milieu studied has a penetrance of 84% in the heterozygous male but is poorly penetrant in the heterozygous

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female (18 %). Obese animals of both sexes are fertile and, in contrast to most other obese mutants, can be used for breeding.

To date, the studies that have been performed on this new mutant relate to its genetics (Wallace & MacSwiney, 1975, 1978). The purpose of the present communication is to present some basic compositional and physiological information including information on the distribution of fat, the size and observable number of fat cells, and on the serum concentration of glucose and insulin. In other strains of genetically-obese rodent hypertrophy of the fat cells is a constant feature and this may be accompanied by hyperplasia (Johnson & Hirsch, 1972). Hyperinsulinemia is also invariably observed in other genetically-obese animals (Bray & York, 1971; Herberg & Coleman, 1977).

The results presented here have been obtained on mice aged 6 months: 4 months is the earliest age at which obese individuals can be differentiated from their lean litter-mates by visual inspection. The group of lean animals will have included both 'normals' and those individuals in which the *Ad* gene is not penetrant. The obese group may contain animals which were either heterozygous, or homozygous for obesity.

EXPERIMENTAL

Animals

The colony of *Ad* mice was established from four breeding pairs obtained from the Department of Genetics, University of Cambridge, in February 1976. The animals were housed in plastic cages in a room maintained at $20 \pm 2^{\circ}$ with a 12 h light – 12 h dark cycle. The colony was fed on Spillers–Spratts Rodent Breeding Diet No. 1 (Spratts Patent Ltd, Barking, Essex) and both food and tap-water were available *ad lib*.

Breeding was usually carried out between one fat and one thin partner when the animals were aged approximately 5 months. This delay in using animals for breeding was necessitated by the need to be certain of the identity of the obese animals. Pairs, once mated, were kept together for the duration of their active breeding life. Litters were weaned abruptly when aged 21 d, and the sexes were caged separately.

Preparation of carcasses

Mice were killed by diethyl ether anaesthesia. They were then weighed, and the body length and tail length measured. The liver and the gastrointestinal tract were each removed, and the former was immediately weighed after excess blood had been absorbed with filter paper. The contents of the gastrointestinal tract were washed out and this organ, along with the liver, was returned to the carcass. This was then stored at -25° to await further analysis.

The processing of the carcasses before the determination of fat was performed essentially as described by Lofti *et al.* (1976). The carcass and organs were cut into small pieces with scissors, and autoclaved at 120°, 15 p.s.i., for 15 min. After cooling, a quantity of distilled water equal in weight to the carcass was added and the mixture was homogenized to a fine suspension using an MSE blade homogenizer (MSE Ltd, Crawley, Sussex). The homogenate was then frozen with liquid nitrogen and freeze-dried to a constant weight. The dried material was stored in a dessicator until required.

In some animals, four major fat depots were dissected before the carcass was processed. These depots were: (1) gonadal, i.e. epididymal in males, parametrial in females; a pair of fat pads in each animal; (2) abdominal, which included mesenteric, perirenal, retroperitoneal and any other adipose tissue lying within the abdominal cavity. (In lean animals these depots were discrete but in obese animals all depots became merged and it was impossible to estimate accurately their relative contributions); (3) hind subcutaneous, which consisted of

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a pair of subcutaneous fat pads extending part way along the abdomen from the hind limbs; (4) interscapular subcutaneous, which included some brown adipose tissue located deep in the interscapular space and capped by white adipose tissue.

The dissected fat was weighed and small samples were taken for determining the cell size and apparent number (see p. 213). The bulk of the fat was then returned to the carcass for analysis as described previously.

Fat analysis

Storage fat was analysed by the Foss-Let Specific Gravity technique, essentially as described by Woodward *et al.* (1976). Samples of the dried carcass weighing approximately 2 g were extracted twice with 24 ml tetrachloroethylene. The two extracts were pooled and made up to 50 ml in a volumetric flask. They were then left to stand at room temperature for 30 min to allow any insoluble material to collect at the surface. The debris was removed with a Pasteur pipette and the specific gravity of the clear solution determined in the Foss-Let 15310 instrument (Foss Electric UK Ltd, The Chantry, Bishopthorpe, York).

Determination of carcass N

A micro-Kjeldahl method was utilized. Approximately 300 mg of the defatted and powdered dry carcass was digested for 2 h with boiling concentrated sulphuric acid to which hydrogen peroxide (oxidizing agent) and selenium (catalyst) were added. The ammonium sulphate formed was determined colorimetrically using a Technicon AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants).

Determination of fat cell size and number

The diameters of fat cells were measured by a modification of the method described by Gurr *et al.* (1977). Pieces of tissue were fixed in (9 g sodium chloride/l) formol-saline (100 ml/l) immediately after dissection and processed within a few days. Slices of tissue, varying in thickness from 100 to 200 μm (depending on the maximum size of the fat cells) were cut with a freezing microtome at $-30°$ (Leitz Kryomat; E. Leitz GmbH, Wetzlar, Germany). Four sections from each specimen were floated on to a drop of physiological saline on glass slides, capped with cover slips and photographed. Because sections were relatively thick and several cells were superimposed an objective lens (magnification $\times 6{\cdot}3$) with a large depth of field was used so that as many cells as possible were in focus. Sections were photographed with an Asahi Pentax camera using Ilford FP 4 (125 ASA) 35 mm film. Correct exposure was determined with an exposure meter attached to one of the eye-pieces of the Reichest binocular microscope. Images were projected on to a white card and from each section ten cell diameters were measured in one direction and then ten in a direction perpendicular to the first. This procedure was adopted to overcome errors that might be caused by polarization of the fat cells. Diameters were recorded in four sections from each tissue site. Results were processed according to Gurr *et al.* (1977). For calibration, a standard microscopic graticule slide was photographed at the same magnification as the cells and the image projected in the same way.

The mass of triglyceride per cell was calculated by assuming that the density of triglyceride was 0·915 (Hirsch & Gallian, 1968) and that the volume of the cell was equal to that of the triglyceride which it contained (Goldrick, 1967; di Girolamo *et al.* 1971). The number of cells in a depot was then derived by dividing the mass of triglyceride in that depot by the mass of triglyceride per cell.

Adipose tissue triglyceride was extracted by grinding the tissue in diethyl ether with an Ultra-turrax homogenizer (Janke & Kunkel K G, Staufen-im-Breisgau, W. Germany). The residue was re-extracted, filtered, the combined solvents removed by distillation, and the

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triglyceride residue extracted and weighed. Preliminary experiments established that quantitative extraction of triglyceride, but no extraction of phospholipid was achieved. The triglyceride content was 724 g/kg wet tissue for lean mice, and 850 g/kg wet tissue for obese mice.

The over-all error in the determination of fat cell size was ±7 %, and the total error in measuring fat cell number was ±9 % (Kirtland *et al.* 1976).

Determination of serum glucose and insulin

Mice of both sexes were taken between 09.00 and 10.00 hours without previous fasting. They were lightly anaesthetized with diethyl ether, decapitated, and blood was collected, and allowed to clot. The cells were sedimented by centrifugation and the serum divided into two portions. One portion was stored at −25° until required for the assay of insulin. The other portion was used immediately for the measurement of glucose.

Insulin was measured by method C of Hales and Randle (1963) with materials from the Radiochemical Centre (Amersham, Bucks) and the Wellcome Foundation (Beckenham, Kent). A human insulin standard was used, and the assay had a coefficient of variation of approximately 10 %. Glucose was measured by the glucose oxidase-peroxidase (GOD-Perid) method with a Boehringer kit (Boehringer Corp. Ltd, London).

Statistical analyses

For most measurements statistical differences between groups were determined using an unpaired Student's *t* test. However, fat cell size and number were compared by analysis of variance of the values which were first transformed to $\log_{10}$.

RESULTS

Body-weight and composition

The weights and body composition of lean and obese animals aged 6 months are shown in Tables 1 (males) and 2 (females). In both sexes the weight of the obese animals was more than 50 % greater than that of the lean. This increase in weight occurred without any change in either the length of the tail or that of the whole body. Obese animals of both sexes showed hypertrophy of the liver, with an increase in the weight of this organ of two thirds compared to the lean animals. The liver size was increased, however, only in proportion to the increase in the total body-weight, for there was no significant difference between the two phenotypes in liver weight:body-weight.

The carcass dry matter DM and the water content were greater in absolute amounts in obese animals of both sexes. The former was increased more than twofold and the latter by approximately one-third. The carcass fat was almost four times greater in the obese than the lean males, while in the females it was increased 5·5-fold. Over half the excess weight of the obese mice was accounted for by fat. The percentage of the body-weight due to fat was increased from just under 9 % in the lean animals of both sexes to over 22 % in the obese males and 29 % in the obese females.

The N content of the 'defatted' DM, which is a measure of total carcass protein, was the same for both lean and obese animals. However, since the 'defatted' DM was increased in the obese compared to the lean by 28 and 22 %, for males and females respectively, the obese animals have an increased protein content (the 'defatted' DM was the difference between the carcass fat and the total carcass dry weight).

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Table 1. *Body composition of male* Ad *mice*

(The results are mean values with their standard errors for eight lean and eight obese mice, except for the tail and body lengths where six mice from each group were used)

	Lean		Obese			
	Mean	SE	Mean	SE		Obese/Lean
Wt (g)	19·04	0·43	29·25	0·86	***	1·54
Liver wet wt (g)	1·164	0·087	1·931	0·114	***	1·66
Liver: body-wt	0·0612	0·0043	0·0657	0·0021	NS	1·07
Tail length (mm)	75	1	75	2	NS	1·00
Body length (mm)	159	2	166	3	NS	1·04
Carcass						
Dry matter, DM (g)	5·90	0·19	12·02	0·49	***	2·04
Water (g)	13·14	0·33	17·26	0·44	***	1·31
Fat (g)	1·68	0·20	6·61	0·42	***	3·94
DM (g/kg)	310	8	410	7	***	1·32
Water (g/kg)	691	8	590	7	***	0·85
Fat (g/kg)	89	10	225	10	***	2·54
Nitrogen in defatted DM (g/kg)	113	2	114	2	NS	1·01

NS, not significant ($P > 0·05$).
*** $P < 0·001$.

Distribution of body fat

The weight of adipose tissue from four depots which were easily removed by dissection was measured with a view to determining whether the extra fat of the *Ad* mouse is distributed throughout the body or whether it is localized in particular regions. These four regions accounted for essentially all the well-defined body fat stores. The results are shown in Table 3. All four depots of dissectable fat were substantially larger in the obese animals. In both males and females, the greatest difference between the lean and obese was in the amount of abdominal fat. The total weight of dissected fat was almost four times greater in the obese males than in the lean males, while the obese females had an 8·7-fold increase over the lean females. Although no accurate determination was made of the amount of brown adipose tissue in the interscapular space, qualitatively it appeared to be similar in lean and obese mice, whereas the overlying white adipose tissue had expanded substantially in the obese animals.

Fat cell size

The size of fat cells was significantly larger in obese animals compared with lean. This was true for all adipose tissue depots examined, although the differences were less in females (2- to 3-fold) than in males (3- to 5-fold) (Table 4). Fat cell size varied according to the site, the internal cells being significantly larger than subcutaneous cells in both males and females (Table 4).

Because the pattern of fat cell sizes in the different depots varied in the same manner in lean and obese animals of both sexes an over-all body fat cell size has been calculated by combining values from all depots (Table 6). Although the mean cell size in lean females is twice that for males, the difference is only just significant ($P < 0·05$) comparing log transformed values.

Table 2. *Body composition of female* Ad *mice*

(The results are mean values with their standard errors for nine lean and nine obese mice, except for the tail and body lengths where six mice from each group were used)

	Lean		Obese			Obese/Lean
	Mean	SE	Mean	SE		
Wt (g)	16·18	0·50	27·11	0·68	***	1·68
Liver wet wt (g)	0·905	0·055	1·525	0·055	***	1·69
Liver : body wt	0·0560	0·0033	0·0568	0·0029	NS	1·01
Tail length (mm)	73	2	70	2	NS	0·96
Body length (mm)	156	2	161	3	NS	1·03
Carcass						
Dry matter DM (g)	5·34	0·24	12·42	0·63	***	2·33
Water (g)	10·84	0·37	14·69	0·38	***	1·36
Fat (g)	1·44	0·14	7·88	0·55	***	5·45
DM (g/kg)	330	10	457	15	***	1·38
Water (g/kg)	670	10	543	15	***	0·81
Fat (g/kg)	89	8	289	15	***	3·25
Nitrogen in defatted DM (g/kg)	112	2	115	1	NS	1·03

NS, not significant ($P > 0·05$).
*** $P < 0·01$.

Table 3. *Weight (mg) of the major dissectable fat depots* from male and female, lean and obese* Ad *mice*

(The results are the mean values with their standard errors for six animals in each group)

	Lean		Obese		Obese/Lean
	Mean	SE	Mean	SE	
			Male		
Gonadal fat	382	92	1203	63	3·15
Abdominal fat	95	26	712	83	7·49
Interscapular subcutaneous fat	311	59	1056	112	3·40
Hind subcutaneous fat	278	63	1114	161	4·01
Total dissected fat	1066	230	4131	351	3·88
			Female		
Gonadal fat	100	33	1742	98	17·42
Abdominal fat	48	25	967	124	20·15
Interscapular subcutaneous fat	222	51	990	139	4·46
Hind subcutaneous fat	158	32	890	83	5·63
Total dissected fat	528	127	4589	345	8·69

All the differences between lean and obese animals were significant ($P < 0·001$).
* For definitions, see p. 212.

Fat cell number

Total body fat cell number was calculated by summing the fat cells in each depot calculated as described in the experimental section. The total number of observable body fat cells as estimated by this method was the same in obese males as in lean males (Tables 5 and 6). The increase in body fat mass in the obese *Ad* male is therefore entirely accounted for by cellular expansion.

This was not so in females. The lean females had a much smaller number of cells than the lean males but obese females had the same number of fat cells as obese males, i.e. more

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Table 4. *Fat cell size (μg triglyceride/cell) in four fat depots* of lean and obese* Ad *mice*

(The results are the mean values with their standard errors, and the number of animals are in parentheses)

Fat depot...	Hind subcutaneous					Interscapular subcutaneous				
	Lean		Obese			Lean		Obese		
Sex	Mean	SE	Mean	SE	*P*	Mean	SE	Mean	SE	*P*
♂	0·070 (6)	0·028	0·296 (6)	0·067	< 0·01	0·047 (4)	0·036	0·207 (6)	0·031	< 0·01
♀	0·139 (5)	0·026	0·197 (6)	0·034	NS	0·097 (5)	0·026	0·258 (6)	0·055	< 0·05
♂ *v*. ♀ *P*	NS		NS			NS		NS		

Fat depot...	Gonadal					Abdominal				
	Lean		Obese			Lean		Obese		
Sex	Mean	SE	Mean	SE	*P*	Mean	SE	Mean	SE	*P*
♂	0·130 (6)	0·045	0·430 (6)	0·043	< 0·001	0·154 (3)	0·068	0·426 (6)	0·033	< 0·01
♀	0·298 (5)	0·082	0·665 (6)	0·079	< 0·01	0·200 (3)	0·070	0·475 (6)	0·080	< 0·05
♂ *v*. ♀ *P*	NS		< 0·05			NS		NS		

NS, not significant ($P > 0·05$).
* For definitions, see p. 212.

than three times as many as lean females ($P < 0·001$). The increased number of cells in obese females was particularly apparent in the abdominal, parametrial and internal depots. There was only a modest increase in the number of fat cells in the interscapular depot of the obese although this region contributed the most cells in lean animals. Thus the increased mass of interscapular fat in obese females was mainly the result of an increase in mean cell mass (2·7-fold). This contrasted with the abdominal depot which increased its mean cell mass only 1·4-fold, but its apparent cell number by 4·7-fold.

Serum glucose and insulin

The glucose and insulin concentrations in serum for non-fasting lean and obese mice are shown in Table 7. The results for males and females have been pooled since no sex differences were apparent. No significant difference between lean and obese animals in the glucose concentration was observed. However, the obese mice exhibited a 5-fold increase in the concentration of insulin.

DISCUSSION

The *Ad* mutation was discovered in a group of wild mice caught in Cambridge in 1971 and was linked to warfarin resistance (Wallace, 1972; Wallace & MacSwiney, 1975). The mutant gene is found at the Gv-2 end of chromosome 7 (Wallace & MacSwiney, 1975). The *Ad* mouse appears to be the first single-gene obese mutation that has been found in a wild rodent, for the other single-gene mutations were discovered in laboratory-bred animals. Polygenic inheritance of obesity in 'wild' animals is well recognized (Bray & York, 1971).

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Table 5. *Fat cell number* (× 10⁶) *in four fat depots* of lean and obese* Ad *mice*

(The results are the mean values with their standard errors, and the number of animals are in parentheses)

Fat depot...	Hind subcutaneous					Interscapular subcutaneous				
	Lean		Obese			Lean		Obese		
Sex	Mean	SE	Mean	SE	P	Mean	SE	Mean	SE	P
♂	3·84 (6)	0·73	3·25 (6)	0·86	NS	7·83 (4)	2·01	5·13 (6)	1·32	NS
♀	0·90 (5)	0·16	4·26 (6)	0·58	< 0·001	2·21 (5)	0·47	3·14 (6)	0·42	NS
♂ v. ♀ P	< 0·01		NS			< 0·02		NS		

Fat depot...	Gonadal					Abdominal				
	Lean		Obese			Lean		Obese		
Sex	Mean	SE	Mean	SE	P	Mean	SE	Mean	SE	P
♂	2·60 (6)	0·44	2·57 (6)	0·40	NS	0·89 (3)	0·28	1·42 (6)	0·12	NS
♀	0·30 (5)	0·07	2·36 (6)	0·24	< 0·001	0·38 (3)	0·09	1·97 (6)	0·42	< 0·05
♂ v. ♀ P	< 0·001		NS			NS		NS		

NS, Not significant (P > 0·05).
* For definitions, see p. 212.

Table 6. *Average body fat cell size and total body fat cell number in lean and obese* Ad *mice*

(The results are mean values with their standard errors, and the number of animals are in parentheses)

	* Cell size (μg triglyceride/cell)					Cell no. (× 10⁶)				
	Lean		Obese			Lean		Obese		
Sex	Mean	SE	Mean	SE	P	Mean	SE	Mean	SE	P
♂	0·089 (6)	0·032	0·340 (6)	0·033	< 0·001	12·1 (6)	2·4	12·4 (6)	2·2	NS
♀	0·184 (5)	0·039	0·407 (6)	0·046	< 0·01	3·6 (5)	0·6	11·7 (6)	1·0	< 0·001
♂ v. ♀ P	< 0·05		NS			< 0·02		NS		

NS, not significant (P > 0·05).
* Arithmetic mean of the individual sites.

The present study has shown that at 6 months of age the body-weight of the *Ad* mouse was approximately 50–70 % greater than in lean animals of the same strain, and that the excess weight is the result of a genuine obesity (Tables 1 and 2). The fat content of the obese animals was four times higher than in the lean for the males, and 5·5 times higher for the females. Of the excess weight of the obese males 48 % was accounted for by fat, with a further 40 % being due to water. The remaining 12 % of the excess weight was caused by an increase in body protein. The hypertrophied liver of the *Ad* mouse could account for approx-

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Table 7. *Concentration of glucose and insulin in serum of* Ad *mice*

(The results are mean values with their standard errors, and the number of animals are in parentheses)

	Lean		Obese		
	Mean	SE	Mean	SE	
Glucose (mg/100 ml)	203·6	8·3 (16)	217·1	7·6 (11)	NS
Insulin (μU/ml)	22·7	2·0 (12)	121·4	39·8 (11)	**

NS, not significant ($P > 0·05$).
** $P < 0·02$.

imately 20 % of this excess protein. In the females, 59 % of the obese animals' excess weight was due to fat, and 35 % to extra water with the remaining 6 % again being due to protein.

The tail length, and the nose-to-tail-tip length were the same in lean and obese animals. This indicates, along with the increase in body protein, that the stunting which is characteristic of obese animals such as the *ob/ob* and *db/db* mice, and the Zucker (*fa/fa*) rat, does not occur in the new mutant: a reduction in body protein is a characteristic of these recessive obese animals.

The results in Table 3 show that all the dissectable fat depots in *Ad* mice of both sexes are larger than the corresponding depots in lean animals. The *Ad* mouse has, therefore, like all other obese mutants with the exception of the New Zealand obese mouse (*NZO*), a generalized increase in body fat. The *NZO* mouse accumulates fat mainly in the abdomen (Crofford & Davis, 1965).

Fat cell hypertrophy is invariably found in genetically obese animals (Johnson & Hirsch, 1972; Johnson *et al.* 1971) and the *Ad* mouse is no exception. Measurement of fat cell mass in *Ad* mice showed that obese animals of both sexes had larger fat cells than lean animals in all sites examined. The trend towards larger cells in all depots of the female mice compared with males, whether lean or obese, has been observed in other strains of mouse (Lemonnier, 1972) and in guinea-pigs (Kirtland *et al.* 1976).

Increased cell numbers in the adipose depots of obese mutants, are not, however, universally observed. In some strains, an increase in the observable fat cell number forms a substantial component of the excessive adipose tissue deposition in the obese animal, whereas in others, hypertrophy entirely accounts for the difference in fat mass. In the *Ad* mouse, there was no difference in the estimated over-all cell number between lean and obese males (Table 6). In females, however, the number of observable cells was 3·3 times higher in the obese than in the lean animals. The observed cell number for obese females was similar to that obtained for lean and obese males.

We prefer the term 'observable' cell number. All the techniques in common use for estimating fat cell number, with the exception of the DNA method (Zingg *et al.* 1962) are dependent on a certain minimal filling of the cell with lipid. Cells smaller than approximately 10–15 μm in diameter are not normally identified (Hirsch & Gallian, 1968; Sjostrom *et al.* 1971). Thus small cells and potential fat cells that have not accumulated any lipid ('empty' cells) would not be counted by the technique and would not be included in estimates of cell number (Widdowson & Shaw, 1973; Gurr *et al.* 1977; Kirtland & Gurr, 1978).

If this hypothesis is correct and there are significant numbers of 'empty' cells present in the adipose tissue of lean animals, our observations may be explained in the following way. In the lean male, the majority of empty cells accumulate lipid and enlarge to a moderate size, whereas in the female, lipid accumulation is restricted to fewer cells which, given similar energy intakes in females compared with males, must expand to a larger size to

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accommodate the available lipid. In obese males, the already partially-filled cells expand further, resulting in the observed increase in size but not number, whereas in obese females, there is a modest expansion of the partially-filled cells accompanied by complete filling of the remaining 'empty' cells. The observed result is an apparent increase in cell number, which might erroneously be taken to be hyperplasia. Additional support for this hypothesis might be gained if the observed fat cell number in lean females increases as they become older and, presumably, fatter. At the same time, there should be an increase in the DNA content of mature adipocytes relative to that in whole adipose tissue.

If the hypothesis of empty cells is untenable, the sex differences in cellularity may have arisen in early life at a critical period for fat cell development (Knittle & Hirsch, 1968) and further longitudinal studies are needed to elucidate the precise mechanism involved. Whichever explanation is correct, the results add further evidence for important sex differences in adipose tissue development which may be hormonally determined.

The serum glucose and insulin measurements shown in Table 7 indicate that in the 'fed state' the *Ad* mouse is normoglycemic, but hyperinsulinemic. The presence of hyperinsulinemia in the face of normoglycemia suggests that the *Ad* mouse is insulin-resistant, assuming that there is no increase in glucose turnover. Both insulin resistance and hyperinsulinemia are features which are common to all genetically-obese animals (Bray & York, 1971). An increased level of insulin is also present in the fasted *Ad* mouse (Trayhurn, unpublished results).

Over all, the results presented here indicate that many of the properties of the *Ad* mouse are similar to those of other genetically-obese rodents. There are, however, several properties of this new mutant which make it an attractive animal for experimental studies on obesity. First, the mutation is dominantly inherited and both obese males and females are fertile and can be used for breeding; this makes the production of obese animals easier than with the commonly-used recessively inherited mutants. Secondly, the obesity develops late, or slowly, and is less marked than in a number of other mutants. Thirdly, obesity has not developed at the expense of stunting which commonly occurs with the *ob/ob* and *db/db* mice, and the Zucker (*fa/fa*) rat. The *Ad* mouse is therefore likely to be a valuable addition to the animals used in obesity research. Its widespread use is likely to depend, however, on the transfer of the mutation to an inbred background.

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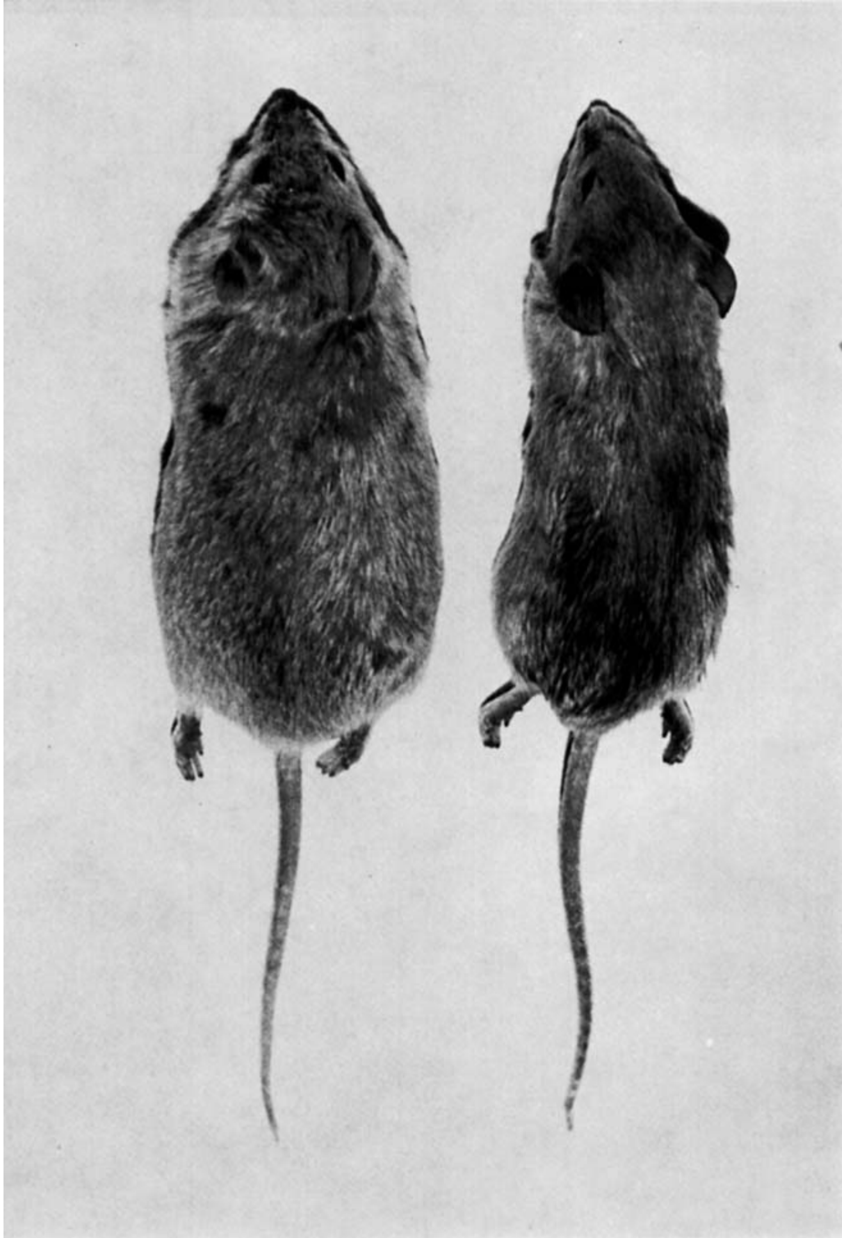

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EXPLANATION OF PLATE

Obese and lean mice aged 6 months from the *Ad* colony (for details, see p. 214).

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