Hereditary Obesity in the Rat Associated with High Serum Fat and Cholesterol.* (27455)

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Extreme obesity has been produced in rats by surgical injury to the hypothalamus(1) and by special (high fat) diets(2). We have recently(3) obtained and studied rats whose genetic constitution is such that they become enormously fat without experimental preparation or the use of special diets. Three low fat diets have been used in production of these rats. Two were commercially pelleted stock diets with 4 to 5% of fat which have also been used in other laboratories. The third was a laboratory made diet based on wheat and milk with about 6% of fat.

The condition is caused by a recessive mutant gene(3) which appeared spontaneously in our 13M strain; the other 5 rat strains maintained in this laboratory have not been affected. The new gene has been named *fa*. Individuals carrying 2 such genes (*fafa*) are noticeably and increasingly fat from the age of 5 weeks, and are designated as "fatties"; litter mates carrying one or 2 normal alleles—*Fafa or FaFa*—are normal in appearance and weight. The average weights at 40 weeks of age for male and female fatties are 800 and 620 g respectively; comparable figures for normal litter mates are 480 and 295. The condition is illustrated by Fig. 1.

The present report deals with observations made on fatties in an attempt to clarify the condition. Since the gene is carried on by breeding heterozygotes (*Fafa*) with a yield of only 25% of fatties per litter, and since heterozygotes must be detected by test mating with known heterozygotes, the supply of fatties has not been great; numbers are, however, adequate to draw several conclusions.

Methods and materials. All rats were kept in screen bottomed cages. Since fatties have been produced regularly on 3 different stock diets it appears unnecessary to detail the composition of each. The one in use at the time the fatties first appeared had the following composition: dried skim milk 15, soybean meal 20, fish meal 10, corn 10, wheat 39, lard 2, alfalfa 2, salt 1, calcium carbonate 0.5, molasses 0.5, plus a complete supplement of vitamins and trace elements. For 2 experiments to be reported here we used other diets. To lessen spillage in determining food consumption of fatties and controls, the following diet of somewhat higher (about 16%) fat content was used: lactalbumin 9, dried skim milk 32, wheat 40, cottonseed oil 14, yeast 2.5, bone ash 2, salt 0.5, plus a complete supplement of vitamins and trace elements. The laboratory-made stock diet referred to above, on which many litters with fatties have been produced, was essentially this same diet (as used in the food intake experiment) except that the version used for stock diet contained only 5% of cottonseed oil. To ascertain whether the normally lean heterozygotes were more susceptible to nutritional obesity than normal homozygote sibs, we used a diet containing 40% of fat (diet FC). Its composition was: cottonseed oil 25, hydrogenated vegetable oil (Crisco) 15, glucose 6, casein 20, soy protein 20, cystine 0.2, yeast 5, whole dry liver 1, salts 6, cellulose (Celluflour) 1.8, and complete vitamin supplement. The salt mixture and vitamin supplements have been described (4); they were here included at a somewhat higher percentage because of the high caloric density of this diet.

Non-esterified fatty acids (NEFA) were determined on fresh plasma according to Dole (5), but using the Nile Blue titration indicator of Gordon(6). The other lipid analyses were all made on aliquots of a Bloor extract (1 volume plasma heated to boiling in alcohol ether 3:1, cooled, diluted to 20 volumes and filtered). For total fatty acids (FA), Bloor extract corresponding to 0.1 ml plasma was evaporated to dryness in a 15 ml centrifuge tube; the material was saponified in hot aqueous alkaline isopropanol, acidified, and the FA extracted into heptane and titrated as in

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the NEFA procedure. Cholesterol was determined by the Liebermann-Burchard reaction in chloroform, without saponification. Lipid P was determined by the Fiske-Subbarow method, after ashing with sulfuric acid and hydrogen peroxide. All determinations except of NEFA were done in duplicate. Blood sugar was determined on tail blood deproteinized by the Somogyi technic, using the Nelson colorimetric micro method.

Hyperlipemia and hypercholesterolemia. The most striking finding in the fatty was an apparently universal massive hyperlipemia leading to very obvious serum lactescence. When a milky serum had been noted in a consecutive series of 11 fatties coming to autopsy, and 17 comparable normals of the same strain did not show the phenomenon, the experiment of Table I was set up. The animals whose blood plasma was analyzed for lipid components were chosen at random from available adult fatties and their normal litter mates. The lactescent plasma contained 10 times as much of total fatty acids as control plasma, about 4 times as much cholesterol and lipid P, and possibly a slightly elevated level of non-esterified fatty acids. This could hardly be an alimentary lipemia since the stock diet is a low fat diet (4%), and Table I shows that the hyperlipemia persisted after 18 hours of fasting. Pooled plasma was subjected to centrifuging, with no noticeable separation of a fatty layer under conditions recommended for separating chylomicrons(7); however one hour at 25,000 g did separate out much of the fat. This suggests that the lipids were present as low density lipoproteins.

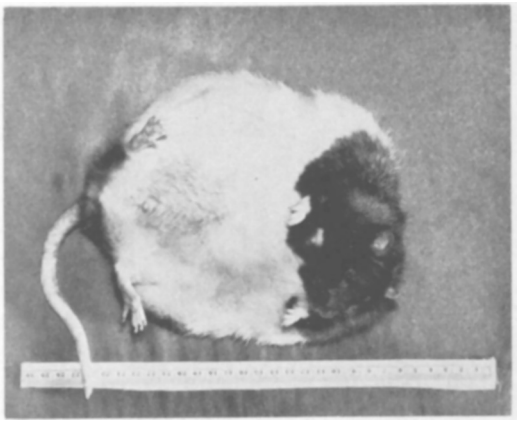

FIG. 1. Male fatty, aged 10 mo, weighing 1035 g. Scale in cm.

TABLE I. Blood Lipids in Fed and Fasted Adults.

	Lactescence incidence	NEFA, μmol	Total FA, mmol	Cholesterol, mg	Lipid P, mg	Est. total lipids, mg
		Per 100 ml heparin. plasma				
		Fed *ad libitum* (stock diet)				
Fatties *(fafa)*	14/14	48	6.00	489	30.8	2,730
		—	8.81	410	25.6	
		65	7.91	218	20.3	
Controls *(Fa—)*	0/20	19	.68	89	6.0	367
		—	.82	95	7.7	
		33	.83	90	6.7	
		Fasted 18 hr				
Fatties *(fafa)*	6/6	—	6.24	350	23.9	1,920
		61	3.54	352	19.6	
Controls *(Fa—)*	0/2	—	.74	93	6.2	329
		64	.58	91	6.0	

Lactescence—a distinctly milky appearance of serum or plasma. FA—fatty acids. NEFA—non-esterified fatty acids. Tabulated figures for NEFA, FA, cholesterol and lipid P represent individual rats; tabulated values for estimated total lipids represent avg of 2 or 3 individuals. Estimated total lipids were calculated as the sum of mg FA (284 times mmol FA), mg cholesterol and 8 times mg lipid P. The quantity 8 times P accounts for the phospholipid molecule exclusive of the FA, since these FA have already been counted in the weight of total FA. Glycerol of triglycerides cannot be calculated, but is a small quantity.

Age ranged from 6 to 12 mo. Of the 10 rats whose blood was analyzed, the first fed fatty and the first fed control were males; the others were females. Of the additional 32 rats observed for serum lactescence, there were about equal numbers of males and females.

TABLE II. Blood Lipids in Young Rats Fed *ad Libitum* (Stock Diet).

N	Age	Lactescence incidence	NEFA, μmol	Total FA, mmol	Cholesterol, mg	Lipid P, mg	Est. total lipids, mg
			Per 100 ml heparin. plasma				
			Fatties *(fafa)*				
3	39 days	0/3	40	1.30	146	9.6	590
2	73 "	2/2	55	2.50	237	11.3	1,035
2	76 "	2/2	60	2.85	313	13.3	1,230
2	81 "	2/2	56	3.22	330	12.3	1,340
3*	6–12 mo	3/3	57(2)	7.57	372	25.6	2,730
			Controls *(Fa—)*				
8	39–76 days	0/8	31(7)	.80(7)	86	6.1	362
3*	6–12 mo	0/3	26(2)	.78	91	6.8	367

* Data of Table I, avg of the fed group.

Figures in parentheses indicate actual number of samples analyzed, when different from number in group.

Approximately equal numbers of males and females were used; no sex differences were observed.

Further work on younger rats is presented in Table II. An analytically detectable hyperlipemia was present at 39 days of age, although not sufficient to produce visible lactescence. By 73 days of age, fat content had increased considerably, and the plasma was now obviously milky. Cholesterol approached the high adult values more rapidly than the other 2 components. The NEFA level characteristic of fed fatties of any age was consistently about twice that for fed normals, and much like that of fasted normals. Conceivably the NEFA rise in the fatties is an artifact. The time between blood collection and getting the plasma into the extraction mixture was about 2 hours (in the cold room), and we have not investigated the possibility of a high rate of spontaneous liberation of free fatty acid from the very high lipoprotein substrate.

Effect of food restriction on fat deposition. At various times a total of 50 male fatties have been fed daily portions of food allowing them to gain weight at the same rate as normal litter mates, usually over the age period 5 to 20 weeks. Such rats have not been available for autopsy as they were involved in a breeding project of overriding priority; male fatties occasionally breed, and we have been much concerned with attempts to increase fertility by suitable food restriction. However even without fat measurements on these fatties fed a limited quantity of food, it is clear from inspection that they were still abnormally fat, with large easily palpable subcutaneous fat pads, and that they were skeletally smaller animals than the normal litter mates of the same weight, with smaller heads, shorter tibias, shorter bodies. Even under conditions of food restriction an abnormally large proportion of the dietary calories goes to adipose tissue, apparently at the expense of the growth needs of other tissues. This situation has also been found in the hyperglycemic obese mouse, where carcass fat analysis verifies an abnormal tendency to lay down fat even under conditions of dietary restriction (8).

Tissue fat mobilization. Fat mobilization from adipose tissues is possible in fatties; how the rate compares with that in normal rats is not known. When totally starved, young 59-day-old fatties used up their entire visible fat before succumbing after 51 days; normal litter mates, with much smaller fat stores, died after 8 days; normal litter mates on a diet exclusively of cottonseed oil lasted 37 days. A few pilot observations were made on NEFA output from slices of fat pad equilibrated with albumin solution, according to the technic of Gordon and Cherkes(9). Not enough data are available for quantitative comparison of fat pad behavior in normals and fatties, but tissue from the fatties was certainly capable of liberating NEFA at quite reasonable rates.

Food intake. It need hardly be said that rat fatties overeat when allowed. Some food intake data are shown in Table III. Female fatties ate much more than normal females

TABLE III. Food Intake of Fatties and Controls.

	Sex	N	Initial Age	Initial W	Daily (14-day period) Gain	Daily (14-day period) Food
Fatties *(fafa)*	♀	4	40	155	5.9	19.0
Controls *(Fa—)*	♂	4	40	150	5.6	15.1
	♀	4	40	119	2.7	11.0
	♀	4	54	157	2.1	12.7

The controls were litter mates of the 4 fatties. The same rats appear on lines 3 and 4. On line 2 we have matched the group of fatties in age and weight but not sex; on line 3 in age and sex, but not weight; on line 4 in sex and weight, but not age.

(lines 1 and 3) and more than normal males also (lines 1 and 2). By chance initial weight and weight gain over the 14-day period were about the same for the female fatties and the male controls; the much larger amount of food needed by the fatties to lay down this weight is obviously due to the higher fat content of the gain in the fatties.

Blood sugar. Values were in a normal physiological range in the fatties; means and standard deviations for fed and fasted adults aged 2 to 10 months were 121 ± 12 (N = 14) and 97 ± 9 (N = 9), with no age trend. Comparable figures for normal controls were 103 ± 8 (N = 16) and 85 ± 12 (N = 5). Values for the fatties appear to be just significantly above control values; larger numbers would be needed to establish the point fully, but since the difference is small the additional labor did not seem justified. There is no *large* hyperglycemic tendency here, and in this respect the rat fatties differ sharply from the obese mice of Ingalls *et al.*(10) which have been much studied by Mayer and others(11), and in which the condition is designated as the hyperglycemic obese syndrome.

Autopsy findings and other observations. Death of fatties is usually preceded by weight loss, but occasionally a fatty may die at its greatest weight. In practically all cases fatties are still very fat at death. The only grossly obvious pathological findings are in the kidney, where there is almost always a severe lesion much like that found by Brobeck *et al.*(1) in rats made obese by surgical injury to the hypothalamus; there is frequently also kidney sand or stones, with hydronephrosis on one or both sides. Livers are frequently moderately fatty. The skin is very tender and cuts easily with scissors; it is easily torn or cut during life, but these injuries generally heal well.

A series of 12 female and 16 male fatties was killed while still gaining weight, and therefore presumably in good condition, for an investigation of organ weights. Relative to organ weights for normals of the same tibia lengths the fatties showed the following weight ratios (*fafa/Fa—*): liver 2.3, kidneys 1.5, heart 1.3, thyroid 2.4, adrenals 1.7, pituitary 1.0, ovaries 1.8, uterus 0.5. Male sex organs were either highly atrophied or about normal in size and appearance. There are definite indications that restricted feeding during the period 6 to 20 weeks has a favorable effect on the male sex organs.

More recently an obviously greatly hypertrophied pancreas of quite abnormal appearance was found in a failing rat killed at 18 months. In a series of 6 somewhat younger rats, 2 showed an enlarged pancreas of normal conformation. In view of the possible role of insulin in obesity, this situation will be explored further. In the hyperglycemic obese mouse there is increased islet tissue without gross hypertrophy of the pancreas(12,13).

Effect of high fat diet on life span and growth. Four fatties raised on the high fat diet *FC* containing 40% of fat, from 4 weeks of age, presented an extremely gross appearance by 18 weeks of age. One rat killed at this age, had heavier fat pads than found in stock diet fatties of the same age. The other 3 fatties were continued on the diet until death. Table IV shows their life span compared with that of fatties receiving stock diet *ad libitum* and that of fatties on a restricted intake of stock diet. A similar effect of food restriction in increasing the life span of obese mice has been noted by Lane and Dickie(14).

Twelve normal litter mates of these fatties were also raised on the high fat diet. Heterozygotes and normal homozygotes are indistinguishable in appearance and growth to a year of age when fed the stock diet(3); it was hoped that they might show different behavior on a high fat diet. When no large difference in growth or apparent fatness were seen by

TABLE IV. Effect of Dietary Fat and Food Restriction on Life Span of Fatties.

	High fat diet *ad lib.*	Stock diet *ad lib.*	Stock diet restricted
Mean life span (days)	270	323	427
Deaths before 1 yr	3/3	14/18	3/27

In the groups fed *ad libitum* males and females were in approximately equal numbers; the restricted group was entirely male. In normal rats males have a shorter life span than females, when compared on the same dietary regimen.

200 days of age, test matings were set up with known heterozygotes. By 300 days of age some obesity was evident, which increased to 450 days when they were autopsied; however none showed anything approaching the obesity of a fatty. Weight data and other measurements made at autopsy are shown in Table V. The percentage values in the Table show that there is a much greater tendency towards overweight in the heterozygotes (*Fafa*) than in the normal homozygotes (*FaFa*). The sole exception to an excellent individual association between obesity and the presence of one *fa* gene is a male classified as a homozygote which was definitely fat. There is a one in 10 chance with the technic used that this was actually a heterozygote (Table V).

In reference to the blood lipids of Table V, although mean differences were in the direction of more plasma lipids in the heterozygotes, these differences were not significant. The striking and significant effect is that of the high fat diet; in all the rats of Table V, whether heterozygote or normal homozygote, plasma fatty acids and cholesterol were raised above normal and for the group as a whole this was a highly significant rise. Normal values (control data shown in Table II) appear to apply equally to both sexes and to the age range of 6 weeks to a year. In the data of Table V, males were more affected by the diet than females. In man, feeding fat regularly causes both a rise in cholesterol, and a greater rise in males than in females, but according to Olson(15), and others(16), neither effect has been found as a result of feeding fat to rats. Possible explanations for our results are: the great duration of fat feeding, age of the rats, strain, presence of liver in the diet.

As to the effect of genetic constitution on nutritional obesity, our experiment suggests

TABLE V. Effects of High Fat Diet on Phenotypically Normal Sibs (*Fafa* and *FaFa*).

Genotype	N	Body wt (mean & % dev.†) at age 42 d	105 d	200 d	300 d	400 d	450 d	Tibia, cm	R.P. + genital fat pads,* g	Total FA, mmol Per 100 ml hep. plasma	Cholesterol, mg plasma
					Females						
Fafa	2	123	233	315	360	442	487	3.92	61	1.29	123
		0%	−2%	+11%	+20%	+37%					
FaFa	2	123	220	275	326	352	345	3.84	37	.99	110
		0%	−7%	−2%	+8%	+9%					
					Males						
Fafa	2	156	388	520	615	692	759	4.45	84	1.59	212
		+7%	+6%	+14%	+25%	+31%					
FaFa‡	3	148	356	493	558	595	622	4.44	55	1.46	191
		+1%	−3%	+8%	+13%	+13%					
	2	152	346	475	525	543	555	4.42	47	1.29	145
		+4%	+6%	+4%	+6%	+3%					

* Comparable wt for fat pads of one male fatty *(fafa)* raised on the high fat diet and killed at 125 days of age was 110 g; this was a much younger and skeletally smaller (tibia 3.75 cm) rat than the 450-day-old rats in the table.

† Percentage deviations of means from values for 46 females or 27 males on stock diet. The stock diet rats were partly *Fafa*, partly *FaFa*; there is no difference in wt on stock diet(3).

‡ The 3 *FaFa* males were very variable, since one got quite fat (450-day wt, 750 g), the other 2 did not. We have therefore tabulated not only the mean of the 3 but also the mean of the 2 non-fat animals separately. It is possible that any single rat classified as *FaFa* on the basis of a test mating producing one litter of 8 phenotypically normal rats may be mis-classified, and actually be genetically *Fafa*. The probability of this occurrence is $(3/4)^8 = 0.1$, or one chance in 10. There is no possibility of the reverse error; any rat classified as *Fafa* must be so.

that the *fa* gene, while fully recessive in its effect on fat deposition and weight on a low fat diet, does predispose in a single dose to a late moderate obesity on a high fat diet. Nutritional obesity produced by high fat diets has been reported by others(2,17). It is clear from Fenton's work(17) that various mouse strains differ markedly in their ability to become fat on a high fat diet. The genetic basis for such differences cannot however be simply presence or absence of a single dose of a gene like our *fa*, because then some individuals in the susceptible strains would carry the double dose of the gene and become fat even on a low fat diet. Probably many genetic loci affect the response to a high fat diet, of which the *fa* locus is only one. Unfortunately the practical problem of improving production of rat fatties by a simple method of recognizing heterozygotes is not solved by use of a high fat diet.

Discussion. The physiological action of the new fatty mutation in the rat seems to be different from that of other known causes of obesity in mice or rats, since serum lactescence has not previously been reported. Some analytical data are available for the hyperglycemic mouse, according to which total lipid level is about 50% above normal and cholesterol is nearly doubled(18,19). These effects are much smaller than we have found in rat fatties. The rat obesity produced by surgical injury to the hypothalamus is also associated with smaller effects; total fatty acids and cholesterol are said to be 1½ to 2 times control levels(1).

The basic phenomenon in the *fafa* condition is apparently a striking error in fat metabolism. 1) There is a great excess of fat (probably low density lipoproteins) circulating in the blood. 2) Excessive fat is deposited in the tissues. Both these changes occur on a low fat diet and even on a restricted food supply. Adequate fat mobilization from the fat depots occurs. The evidence suggests excessive production of fat in the body, and clearly suggests excessive hepatic output of lipoproteins, since serum lipoproteins are made by the liver. Livers in the *fafa* rats are extremely large; some have the appearance of fairly normal liver tissue, some have a moderately fatty appearance.

The status of the pancreatic islet tissue remains to be investigated. The hyperglycemic obese mouse apparently shows indications of excessive production of both insulin and glucagon(12,13). Glucagon has the ability to clear alimentary lipemia in man(20), in addition to its function of raising blood sugar. Conceivably the large and, at first sight etiologically important, differences between our fatties and the hyperglycemic obese mice in respect to both blood sugar and blood lipids, may be referable to a difference in ratio of insulin to glucagon secretion.

There are several forms of hereditary fatness in mice of which 2 are the obese (*obob*) of Ingalls *et al.*(10) and the adipose (*adad*) of Falconer and Isaacson(21). It appears that the rat obesity described here (*fafa*) is the only such condition found so far in rats. The "Yale" rat is sometimes described as a case of hereditary obesity(22). However, as can be seen from Deuel's(23) discussion of carcass fat in rats, fat content increases faster than body weight during growth; similarly when rats are selectively bred for large and small body size(24) fat content is greater in large bodied than in small bodied rats(25). The Yale rats are of distinctly larger average size than most laboratory rats, and much larger than the controls (Wistars) with which they were compared in fat content by Harned and Cole(26). Maximum fat content of the Yale rats on stock diet was well under 20% (26,27), as against the 40 to 50% levels in obese mouse strains and in our fatties. Twenty percent of fat does not make for very great obesity, and the Yale rat is a normal looking animal.

Summary. A rat mutation leading to obesity noticeable by 5 weeks of age and produced on a low fat stock diet is described. It is associated with approximately normal blood sugar levels, but with a spectacular hyperlipemia, involving a 10-fold rise in total fatty acids, 4-fold rise in cholesterol and lipid phosphorus, and a milky appearance of the blood serum.

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Effects of Sodium Phenylpyruvate on Brain Amino Acids.* (27456)

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In analysis of the free amino acids of serum in phenylketonuric subjects it has been noted (1,2) that along with a high level of phenylalanine, there is a decrease in concentrations of most of the other amino acids. Glycine and histidine, however, are present in normal and excess amounts respectively. Similar data have been obtained in this laboratory(3). It would appear, then, that the high level of phenylalanine is associated with a depression in concentration of other amino acids.

Such studies, therefore, imply a generalized amino acid involvement in phenylketonuria. To examine this concept further, the effects of sodium phenylpyruvate (the characteristic excretory product of the phenylketonuric subject) on the free amino acids of rat brain were investigated.

Methods. Rats (Sprague-Dawley 150-200 g) were sacrificed by dislocation of the neck. After decapitation the brain was removed and separated into hemispheres. Each hemisphere was sliced as thinly as possible by hand in the cold room, weighed, and transferred to Warburg flasks. Incubation in air in the presence of glucose (0.01 mM) and Krebs-Ringer phosphate was carried out for one hour at 37°C. Sodium phenylpyruvate (0.0018 mM) dissolved in the buffer was added to the experimental flasks.

At the conclusion of the experimental period, the medium and tissue were separated

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