

## Analysis of lines of mice selected on fat content. 4. Correlated responses in growth and reproduction

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### Summary

Lines of mice have been selected for 32 generations for either high or low fat content, resulting in a threefold divergence in the selection criterion (estimated fat content of males at 14 weeks of age). Male mice from both lines were dissected at a series of ages between 4 and 26 weeks and the following traits measured or estimated: body weight, fat content, lean weight, and the weights of several fatpads and internal organs. The lines appeared to have a similar underlying lean weight upon which the Fat line accumulated fat at a faster rate. This accumulation continued unabated in the Fat lines for at least 26 weeks but had effectively ceased by 8 weeks of age in the Lean. The liver and kidneys were slightly larger in the Fat line but there were no differences in the weights of heart, lung or spleen. This detailed phenotypic description of the lines complemented previous reports describing correlated changes in their physiology. The threefold divergence in estimated fat content was less than that in one of its component traits, growth of gonadal fatpad, but was greater than the divergence in other physiological indicators, i.e. the activity of lipogenic enzymes *in vitro* and direct measurement of lipogenic flux. Testis size in the Fat line was consistently lower than in the Lean although the Fat line was slightly more fecund, apparently due to a higher prenatal survival rate.

### 1. Introduction

This study investigated lines of mice set up to examine the consequences of long-term selection on a criterion of fat content. Substantial responses in the selection criterion (estimated carcass fat content in males at 14 weeks of age) had occurred in the lines with little apparent difference in other growth characteristics such as lean mass (Hastings & Hill, 1989). Basic metabolism and its physiological control appear similar in all mammalian species (Prosser, 1973), so the results of mouse experiments should be relevant to commercial species. The degree of obesity in the Fat line was similar to that associated with single gene mutations (Johnson & Hirsch, 1972; Festing, 1979). This allowed comparison of similar phenotypes produced by different genetic mechanisms, i.e. single gene mutation vs. polygenic segregation.

Advances in molecular biology provide the opportunity to directly alter the genome of commercially

important species. Several attempts have been made to alter livestock production traits, particularly by introducing additional growth hormone genes (e.g. Polge *et al.* 1989; Pursel *et al.* 1989) in an attempt to improve growth rate. These attempts have been largely unsatisfactory due to extensive pleiotropic effects. A greater understanding of physiological changes associated with a specific production trait (such as fat content) may provide information on potential sites of genetic manipulation which may not incur widespread pleiotropic effects. The physiology and biochemistry of these lines have been the subject of several investigations into (i) the activities of enzymes producing NADPH, an essential cofactor in lipogenesis (Asante *et al.* 1989); (ii) the activities of enzymes directly involved in lipogenesis (Hastings & Hill, 1990); (iii) estimates of lipogenic flux *de novo* (Asante *et al.* 1991); (iv) the growth of adipocytes (Sinnott-Smith & Waddington, pers. comm.), and (v) the effects of dietary manipulation (Moruppa *et al.* 1990). A detailed investigation into growth of these lines provided a greater understanding of the phenotype resulting from long-term selection on fat content. This has improved the knowledge of the phenotypic

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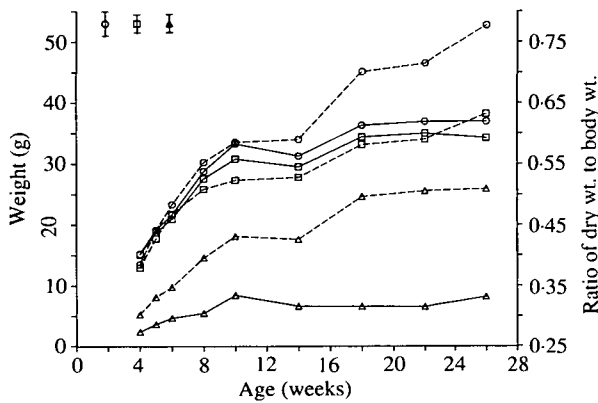


Fig. 1. Body weight (g), water weight (g), and the ratio of dry weight to body weight in males. The maximum S.E. is shown on the key as a  $\pm 1$  S.E. error bar. —, Fat line; —, Lean line; ○, body wt.; □, water wt.; △, dry wt./body wt.

changes associated with the physiological divergence known to have occurred in these lines.

The origins and maintenance of the lines were described by Sharp *et al.* (1984). The original selection criterion was the ratio of gonadal fatpad weight (GFPW) to body weight (BW) in males at 10 weeks of age and selection was practised in three replicates derived from the same base population. The selection criterion was changed at generation 20 to the ratio of dry carcass weight (DW) to BW in males at 14 weeks of age and the three Fat replicates were crossed to form a single line as were the three Lean replicates. Fat content is strongly negatively correlated with carcass water content (Rogers & Webb, 1980; Eisen & Leatherwood, 1981), a relationship confirmed in these lines by analysis of carcass components of 10 week old males at generation 20 (Hastings & Hill, 1989) giving an estimate of the percentage fat as  $113 \cdot DW/BW - 30 \cdot 2$ . At the time of this experiment a further 12 generations had elapsed, resulting in DW/BW values of 0.43 and 0.32 in the Fat and Lean lines corresponding to estimated fat contents of 18% and 6%, assuming the relationship between water and fat content is the same at 10 and 14 weeks of age.

The characters examined were (i) changes in body weight and internal organ growth, (ii) changes in water content and thus predicted lean mass and carcass fat content, and (iii) relative growth rates of individual fat deposits. The Fat lines have slightly larger litter sizes than the Lean lines so the following traits were investigated in an attempt to elucidate its physiological basis: (i) ovulation rate, (ii) pre-natal embryo survival and, by crossing lines, (iii) the effects of embryo genotype.

## 2. Methods

### (i) Growth characteristics

Male mice from generation 32 were kept in groups of six after weaning at three weeks of age and dissected

at 4, 5, 6, 8, 10, 14, 18, 22 and 26 weeks of age. Eight individuals from the Fat line and six from the Lean line (which was less fertile) were examined at each age. An exception occurred at 14 weeks of age (the age of selection) when males mated in the main pedigree lines were routinely killed. This resulted in 48 from the Fat line and 42 from the Lean being available for dissection. As before, these had been kept in groups of six after weaning but at 12 weeks of age were individually housed, each with a single female, to maintain the line. It was assumed that the two week mating period would have a negligible effect on growth but, as will be described later, subsequent results proved otherwise.

Individual mice were killed by inhalation of diethyl ether, weighed and the following organs dissected and weighed: testes, kidneys, liver, lungs, heart and spleen. Three fat deposits were dissected and weighed: gonadal fatpad, hindleg fatpad and shoulder fatpad. The organs and fatpads were re-inserted into the body cavity and the carcass freeze-dried and subsequently weighed.

### (ii) Fertility traits

Animals were taken from generation 34, and control animals were obtained from a contemporaneous unselected replicate. These were mated at ten weeks of age, in a complete diallel cross with six matings in each of the nine combinations. Females were killed 17 days after appearance of the last vaginal plug and dissected as described by Brien *et al.* (1984). The numbers of corpora lutea on the ovaries were counted as were the numbers of moles, resorptions and live embryos present in the uterus. The number of corpora lutea indicated the number of eggs released from each ovary, and the number of moles plus resorptions indicated the number of prenatal deaths. Data collected from each side of the reproductive tract were pooled.

## 3. Results

Changes in body weight, water content and DW/BW are shown in Figure 1. The lines differed in body weight and DW/BW with only a small divergence in water content, indicating that the divergence in body weight was primarily due to differing amounts of fat deposited on a similar lean mass. Overall fat deposition in the Lean lines had effectively ceased by 8 weeks of age while continuing to 18 weeks of age in the Fat line. This interpretation was supported by the growth curves of individual fat deposits shown in Figure 2 which suggested that deposition had ceased by age 8 weeks in the lean line while continuing for at least 26 weeks in the Fat line. The reason why overall deposition had apparently ceased in the Fat line by 18 weeks of age while growth of individual deposits continued unabated was probably due to measurement error of overall fat (of which the individual deposits are

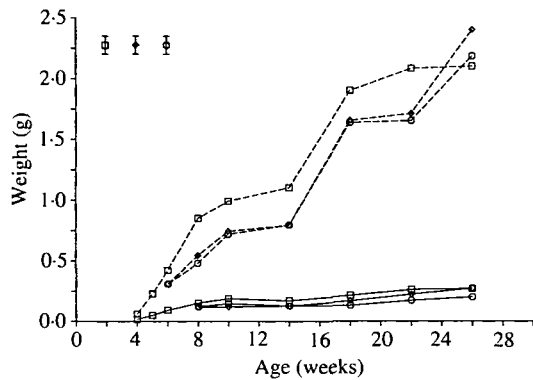


Fig. 2. Weight of gonadal fatpad (GFP), hindleg fatpad (HFP) and shoulder fatpad (SFP) in males. The maximum s.e. is shown on the key as a  $\pm 1$  S.E. error bar. ---, Fat line; —, Lean line;  $\square$ , GFP;  $\circ$ , HFP;  $\diamond$ , SFP.

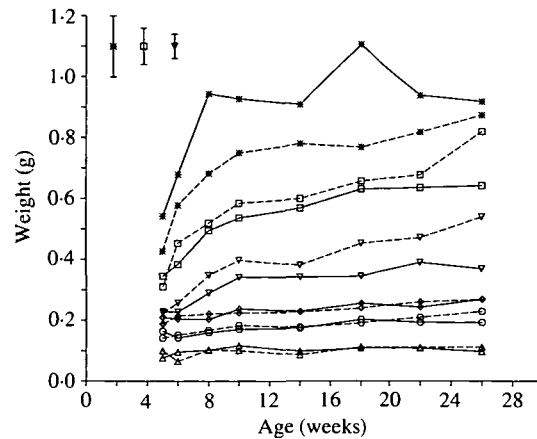


Fig. 3. Weight of testes, kidney, liver, lung, heart and spleen in males; testis and kidney weight are scaled for clarity. The maximum s.e. for testes, kidney and liver are shown on the key as a  $\pm 1$  S.E. error bar. ---, Fat line; —, Lean line; \*, testes ( $\times 4$ );  $\square$ , kidney;  $\nabla$ , liver ( $\times 0.4$ );  $\diamond$ , lung;  $\circ$ , heart;  $\triangle$ , spleen.

a small proportion) rather than a redistribution of fat within the carcass. Changes in organ weight are shown in Figure 3: a positive correlated response was noted in kidney and liver weight, a negative correlated response in testes weight, and no correlated response in lung, heart or spleen weight.

Results from the diallel cross used to investigate components of fertility are shown in Table 1. One Lean dam crossed with a Control sire produced thirteen corpora lutea of which twelve were resorbed

and one was unaccounted for. This dam was regarded as atypical and omitted from the data shown on Table 1, and from subsequent analysis. There appeared to be no significant difference between lines in the number of corpora lutea observed on the ovaries. Sires and dams from the Lean line were associated with the largest number of moles and resorptions and conse-

Table 1. Fertility traits in the diallel cross of Fat, Control and Lean lines. Numbers of families and mean numbers of corpora lutea, embryos alive at day 17 and prenatal survival from corpora lutea to live embryo at day 17, and standard error (S.E.) of cell means

Dam	Sire			Total	S.E. of cell mean	Fat dam- Lean dam ( $\pm$ S.E.)	Heterosis ( $\pm$ S.E.)
	Fat	Control	Lean				
	No. of families						
Fat	6	6	5	17	—	—	—
Control	6	6	6	18			
Lean	6	5	5	16			
Total	18	17	16	51			
	Corpora Lutea			Mean			
Fat	12.00	10.67	11.20	11.29	0.78	-0.01	-0.46
Control	10.00	10.00	10.83	10.28		(0.26)	(0.18)
Lean	11.50	10.60	11.80	11.30			
Mean	11.17	10.42	11.28	10.96			
	Alive at day 17						
Fat	10.17	10.33	10.20	10.23	0.89	0.63	0.83
Control	9.50	8.50	10.50	9.50		(0.30)	(0.21)
Lean	11.00	8.80	9.00	9.60			
Mean	10.22	9.21	9.90	9.77			
	Prenatal survival						
Fat	0.84	0.97	0.92	0.91	0.05	0.06	0.12
Control	0.95	0.85	0.97	0.92		(0.02)	(0.01)
Lean	0.96	0.83	0.76	0.85			
Mean	0.92	0.88	0.88	0.89			

quently had the lowest prenatal survival rate. In contrast, sires and dams from the Fat line were associated with the lowest numbers of moles and resorptions and therefore had the highest prenatal survival rate. This suggested that the slight differences observed in litter size were due to differential survival of eggs and zygotes. Heterosis was measured as the mean value of pure-bred (i.e. Fat  $\times$  Fat, Control  $\times$  Control, and Lean  $\times$  Lean) minus the mean value of cross-bred (i.e. Fat  $\times$  Control, Fat  $\times$  Lean, Control  $\times$  Lean, and their reciprocals). There appeared to be heterosis for prenatal survival and number alive at day 17.

#### 4. Discussion

The lines diverged in body weight and the ratio of dry weight to body weight at all ages whereas their water content (in units of weight) was similar. The weight of water in the carcass is an indicator of lean mass and the growth of estimated lean mass with age appeared similar in both lines.

Murine growth typically exhibits increases in body weight, lean mass and fat content up to maturity at about 10 weeks of age. These characteristics were noted in the Lean lines. In the Fat lines, growth of lean tissue (as measured by weight of water) appeared to cease around ten weeks of age whereas body weight and the ratio of dry weight to body weight continued to increase. The simplest explanation for these characteristics is the continued deposition of fat on a near-constant 'mature' lean mass as suggested by Figures 1 and 2. This accords with previous studies on carcass composition in generations 7, 14 and 20 (Sharp *et al.* 1984; Bishop & Hill, 1985; Hastings & Hill, 1989), indicating that the differences between the lines at 10 weeks were almost entirely attributable to differential deposition of fat on a similar lean mass.

There was a decrease in body weight, lean mass, ratio of dry weight to body weight and growth of individual fat deposits at 14 weeks of age in both lines (Figures 1 and 2). This was presumably an environmental effect since, as explained in the methodology, mice examined at this age had been mated over the previous two weeks while those examined at other ages had been kept in groups of six in stock cages. These changes in growth may therefore have been a result of stress, hormonal changes, or increased reproductive effort over the mating period. A subsequent experiment supports this interpretation. Seventy one males from the Fat line and 61 from the Lean line were kept in groups of five or six in stock cages; at 12 weeks of age 43 from the Fat line and 35 from the Lean line were removed and individually caged with single females. When killed at 14 weeks of age the ratio of dry weight to body weight in the Fat lines was 0.486 in the unmated males and 0.468 in the mated (S.E. of each group was 0.006), equivalent to an estimated 8% decrease in total carcass fat content;

there appeared to be no effect of mating in the Lean line.

The lines showed large differences in growth rates of individual fat deposits. Deposition was more rapid in the Fat line and continued over the whole study period (26 weeks) whereas in the Lean line it had effectively ceased by 8 weeks of age. The relative growth of the three fat deposits appeared unchanged, an interesting result as the lines were selected on the ratio of gonadal fatpad to body weight during the first 20 generations of selection and analysis of the original lines at generations 20 and 29 had suggested the proportion of total fat contained within the gonadal fatpad had been altered (Hastings, 1989; Hastings & Hill, 1989). Growth in the three fat pads was similar (Fig. 2) suggesting their growth was regulated by the same set of genes, distinct from those which regulate growth in other sites such as subcutaneous deposits. This accords with the results of Eisen (1987) who obtained genetic correlations between GFPW and hindleg fatpad weight not significantly different from unity in lines divergently selected on the ratio of GFPW to body weight in male mice at 12 weeks of age. A subsequent investigation into the growth of fat deposits in these lines (Prasetyo & Eisen, 1989) showed that fat deposition in the lean lines ceased around 9 weeks of age, similar to our Lean Line (Figs. 1 and 2) but that deposition in the fat lines ceased at around 12 weeks of age, the age of selection, in contrast to our Fat line in which fat deposition continued unabated until at least 26 weeks of age.

Growth of lung, spleen and heart were similar in both lines. The increased weight of liver and kidneys in the Fat line may have been a result of increased food intake because these organs represent important sites of metabolism and waste excretion. They also contain significant amounts of lipid so divergence in intraorgan fat content may have contributed to the divergence in weight. Increased liver weight is also noted in rodents whose obesity is due to a single gene mutation (Hems, 1979). Liver is a major site of lipogenesis in mice (22% of lipogenesis *de novo*; Holland & Cawthorne, 1981) so indirect selection on its weight cannot be discounted and its weight had diverged by around 12% at 5 and 10 weeks of age.

Sinnett-Smith & Waddington (pers. comm.) measured the size and number of adipocytes in the gonadal fatpad of male mice from these lines at ages 4, 5, 7.5 and 10 weeks at generation 27 and attributed the divergence in GFPW to changes in volume rather than the number of cells. Growth of mean adipocyte volume in the Fat line was nearly linear, increasing by 54 picolitres (pl) between 4 and 7 weeks and by 67 pl between 7 and 10 weeks of age. Growth in the Lean line was slower and had effectively ceased by 7 weeks of age: adipocyte volume increased between 4 and 7 weeks by 11 pl and between 7 and 10 weeks fell by 1 pl. As there was no change in cell number these data suggest a fivefold divergence in the growth of GFP

Table 2. The relationship between the activities of enzymes (expressed per mg soluble protein) involved in the production of NADPH, lipogenesis, and related lipogenic functions; observed lipogenic flux; and growth of GFP. These traits are expressed as the ratio of Fat/Lean in GFP and liver tissue at 5 and 10 weeks of age

	Enzyme <sup>1</sup>	GFP		Liver	
		5 wks	10 wks	5 wks	10 wks
NADPH <sup>2</sup> producing enzymes	G6PDH	1.31	1.05	1.56	0.99
	6PGDH	1.11	1.48	1.43	1.16
	ME	2.25	2.26	1.26	1.17
	IDH	1.27	1.47	1.15	1.06
Lipogenic <sup>3</sup> enzymes	ACL	3.53	1.53	1.52	1.05
	ACC	2.37	1.52	1.12	0.97
	FAS	2.52	1.46	1.21	1.04
Related <sup>3</sup> enzymes	MDH	1.09	0.81	0.99	1.02
	PK	1.38	0.85	1.11	1.03
Flux <sup>4</sup>		1.52	1.69	1.18	1.20
Growth of GFP <sup>5</sup>		5.0	∞	—	—

<sup>1</sup> The enzyme abbreviations: G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; ME, malic enzyme; IDH, isocitrate dehydrogenase; ACL, ATP-citrate lyase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthetase; MDH, cytoplasmic malate dehydrogenase; PK, pyruvate kinase.

<sup>2</sup> Asante *et al.* 1989.

<sup>3</sup> Hastings & Hill, 1990.

<sup>4</sup> Asante *et al.* 1991. This ratio is the mean of the ratios obtained using three differently labelled precursors.

<sup>5</sup> This study; growth is expressed per unit time and its ratio becomes infinite at 10 weeks of age since growth has ceased in the lean line.

between the lines. The data used to generate Figure 2 can be used to calculate the growth of GFP over the period 4 to 6 weeks of age: this was 0.19 gw<sup>k</sup><sup>-1</sup> and 0.04 gw<sup>k</sup><sup>-1</sup> in the Fat and Lean lines respectively, which corresponds almost exactly to the fivefold divergence noted by Sinnott-Smith & Waddington.

These lines have been the subject of several studies designed to elucidate the physiological basis of the phenotypic divergence in fat accretion. The magnitude of the threefold divergence in estimated total carcass fat content can be compared with that of an individual component of total fat content (namely growth of the GFP), and with several other physiological indicators as summarized on Table 2. When arranged in order of magnitude, the divergence in growth of GFP > the divergence in estimated fat content > the divergence in the activities of lipogenic enzymes > divergence in lipogenic flux, and emphasizes the complex nature of composite traits such as carcass fat content. The enzyme activities referred to in Table 2 were the maximum activities ( $V_{max}$ ) obtained *in vitro*. Most enzymes are modulated *in vivo* by mechanisms such as substrate activation, feedback loops, and hormonal modification so their activities *in vivo* may differ markedly from those measured *in vitro*. The results of direct measurement of flux may need to be explained by such modulation *in vivo* for two reasons. Firstly, the activities of the three lipogenic enzymes (ACL,

ACC, FAS; Table 2) which carry the flux from citrate to fatty acid differ by a minimum 2.4 fold in GFP at 5 weeks of age whereas flux *in vivo* appears to differ only 1.5 fold. Secondly, citrate is the direct precursor of fatty acids but both the rate of lipogenic flux and its divergence was less using citrate as a precursor than when using acetate (Asante *et al.* 1991).

The results summarized on Table 2 are relevant for attempts to improve livestock by selection on 'indicator' traits (these traits are genetically correlated to the desired phenotype and may be used as a selection criterion if it is difficult or expensive to measure directly the desired phenotype, or if the indicator trait can be measured at an earlier age, or if the desired trait is sex-limited as, for example, ovulation rate). The results described above have two implications for indirect selection using an indicator trait. Firstly, a carefully chosen sub-component of the phenotype (in this case growth of GFP between 4 and 6 weeks of age) may act as a good indicator. Secondly, a more obvious composite physiological trait (such as lipogenic flux measured *in vivo*) may be no better than one of its component enzyme activities measured *in vitro* (enzyme activities are difficult to measure *in vivo*). These results suggests that a large number of traits may need to be investigated and combined with a detailed understanding of its underlying physiology before a suitable indicator trait is chosen.

The results of the fertility study were similar to those reported for the replicated lines at generation 10 (Brien *et al.* 1984). They obtained values for the Fat, Control and Lean lines of 13.4, 13.5 and 13.4 respectively for corpora lutea, 11.0, 10.8 and 10.4 respectively for numbers alive in the primiparous litter and 0.83, 0.80 and 0.79 respectively for prenatal survival rate. Similar results were noted in the same lines at generation 13 (Brien & Hill, 1986) (the slight differences in the values between lines can be attributed to drift within the replicates). Eisen (1987) selected on carcass fat content using either the ratio of gonadal fatpad weight to body weight (as used during the first twenty generations of selection on these lines), or the ratio of hind carcass weight to body weight and reported a similar small decrease in litter size in the lean lines. The small increase in pre-natal survival in the Fat line is also apparent in pigs. Bazer *et al.* (1988) and Haley & Lee (1990) reported higher pre-natal survival in a fat (Meisham) compared with a leaner (Large White) breed; they also noted that pre-natal death appears to be the factor limiting attempts to increase porcine litter size.

There was a correlated response in testis weight, mice from the Fat line having smaller testes. This may be attributed to indirect selection on testis weight for the following three reasons. Firstly, male hormones, particularly testosterone, are known to reduce fat content (Schanbacher, 1984). Secondly, the reduction in fat content noted at 14 weeks of age in Figure 1, and the subsequent experiment comparing mated with unmated males, suggested that a period of mating reduces fat content; decreased testis size may reduce the amount of hormone secreted or the amount of effort put into reproduction. Thirdly, Hill *et al.* (1990a) derived lines of mice selected on testis weight from the same base population used to produce the Fat and Lean lines described here; they reported a negatively correlated response in fat content. Land (1973) suggested that testis size may be an indirect indicator of ovulation rate as the same range of hormones are involved in gonad development in both sexes and a positive correlated response to selection on testis size was subsequently observed by Islam *et al.* (1976) and Hill *et al.* (1990b) for ovulation rate and litter size, respectively. The changes in ovulation rate observed in the Fat and Lean lines were in the opposite direction to that expected from the changes in testis weight, thus other factors associated with fat content appeared to be involved in determining ovulation rate.

Heterosis was neither expected, nor observed, for ovulation rate since it is a trait of the dam, heterosis only becoming apparent in later generations when the dams are heterozygous (Roberts, 1960; Bhuvanakumar *et al.* 1985). There was, however, heterosis for prenatal survival and thus for the number alive at day 17 although these effects were not large despite inbreeding coefficients of 0.19 in each line. The

differences in litter size therefore appeared to be a result both of maternal effects (mothers selected for low fat had increased zygote mortality) and of the zygotes' own genotype (hybrid zygotes had the highest survival). The magnitude of this correlated change in litter size was small relative to the threefold divergence in estimated fat content. This observation may be relevant to commercial livestock production (where fat content is an important economic characteristic) as it suggests that polygenic divergence in murine fat content need not incur a significant reduction in fecundity.

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