Theoretical and Applied Genetics 48, 95-98 (1976)

The Activity of Certain Enzymes in the Liver of Mice, Selected for Weight Gain

M. Kownacki, A. Kołataj, J. Dembowski, E. Gebler and A. Guszkiewicz

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Mroków (Poland)

Summary. Examining the weight gains of mice in selected and nonselected lines maintained on a low (10%) and high (20%) protein diet, and of their "crossbreds", it was ascertained that the highest values occurred in selected lines maintained on a high protein level and the lowest in "crossbreds".

Analysing the enzyme activity - aldolase, aminotransferase AspAT and AlAT - in the liver of these animals, it was observed that selected mice maintained on either of the protein levels demonstrated usually values significantly lower than for the nonselected ones.

1. Introduction

Selection with a view to increasing weight gains of mice was carried out for several generations and resulted in considerable differences between animals selected and those nonselected. Several authors (Timon, Eisen and Leatherwood 1970; Roberts 1973; Brown and Frahm 1973) have indicated that with increased body weight gains, in selected mice, there is a slight improvement in appetite, a higher feed conversion etc. Timon, Eisen and Leatherwood (1970) also stated that in the carcasses of selected mice the content of protein, ether extract, ash and water is higher than in nonselected animals. Baker and Chapman (1975), conducting similar investigations on rats, demonstrated that in lines selected for high weight gain the carcass contained more water and less fat than in the control, nonselected animals. Gall and Medrano (1974) stated that animals from a selected line achieved higher body weights, by more efficient carbohydrate metabolism, than individuals from nonselected lines.

Kownacki, Keller and Gebler (1975) demonstrated that selection for larger weight gains in mice also caused differentiation of the animals as regards metabolism. An inversely proportional relation was demonstrated between the rate of gain of young mice and their metabolic rate at the age of 5 weeks. There were also indications that the kind of diet influenced metabolic rate in mice.

The present authors have not found literature referring to the variability, in mice or any other animal, of enzyme activity caused by selection, although the performance of certain physiological indicators has been observed for inbred lines. Thus, for instance, Badrutdinov and Elkin (1969) found differences in the intensity of energy metabolism, in various lines of rats and Elkin and Fedorov (1969) found differences in certain nervous processes.

Makarova (1967) observed that inbreeding causes certain deviations from physiological norms. Eremiejev (1969) published interesting observations on the weight of the brain in rats and of its acetylcholine content; he found differences between lines. Earlier scientists had already proposed selection of rats destined for laboratory investigations on the basis of biochemical indicators (Kyle and Chapman 1953, Weir and Clark 1955, Roderick 1960).

The experimental scheme proposed in the present work for the observed variations in weight gain of selected mice was used also for a parallel analysis of the activity of three model enzymes in the liver of those animals.

2. Material and Methods

Studies were conducted on mice not selected (control) and selected for 10 generations with a view to achieving large weight gains between the 21st and 42nd day of life. The experiment covered 5 lines of mice. Two lines were maintained on a diet containing 20% of crude protein - one (H) was selected for large weight gains, the other was the control (Hc), not subjected to selection. The next two lines of mice received a diet containing 10% of crude protein and similarily one (L) was selected for large weight gains, while the other constituted a control group (Lc). The fifth line was formed in the third generation from selected female mice maintained on a low protein diet and selected males receiving a high protein diet. Thus the female progeny from this line was mated in every generation according to an upgrading system. This line remained permanently on a low protein diet. The fifth line was

similar to an arrangement often used in mass breeding - i.e., mating elite males maintained in good conditions with females from range breeding, often in very poor conditions.

In all lines, food was given ad libitum. The investigated lines were similar numerically and included 40 parental pairs (10 pairs in 4 replications) and their progeny. The selection was carried out within litters and mating of related animals was avoided in order to prevent an increase of inbreds.

The mice were maintained in a mice room at a temperature of 20-22°C, with light for 12 hours and relative humidity about 60%. All mice were weighed on the 21st and 42nd day of life.

The mice used for the examination of enzyme activity were weighed also at age 130 days. In the tenth generation of each line, 23-25 mice at age 120-130 days were taken for the estimation of enzyme activity.

These mice were decapitated and the liver was taken out whole; after separating the gall bladder, the liver was homogenised in cooled acetone and the solution obtained filtered. The precipitate was washed three times with cooled acetone and dried in an exiccator in the presence of $CaCl_2$. The dried samples were stored in closed glass tubes at -27°C.

Before the determination of enzyme activity, 10 mg of the powder was homogenised with 5 ml of a 0.1 M buffer solution ($NaHP0_4 \times 12H_20 + KH_2P0_4$) at pH 7.4 and centrifuged for 10 minutes.

Next, 1 ml of the supernatant was diluted in 4 ml of the mentioned buffer solution. The activity of aldolase (FDPA, 4.1.2.13) was determined in the obtained solution by the method of Sibley-Lehninger, and the activity of aminotransferases AspAT (2.6.1.1) and AlAT (2.6.1.2) by the method of Reitman and Frankl (Krawczyński and Osiński 1967).

The activity of individual enzymes, determined in units specific for the method applied, was calculated for 1 mg of protein applying Lowry's method. This indicator is used in the whole work.

The results obtained were subjected to a statistical analysis with the application of Duncan's test. The significance of differences is presented in tables.

3. Results

As a result of selection continued through 10 generations (Table 1) the highest weight gains were obtained in the selected line receiving a high protein diet, and the lowest in the line made up of selected females maintained on a low protein diet and mated with selected males receiving a high protein diet. The differences in weight gains between the three selected lines were highly significant statistically. Highly significant differences were found also between the selected and control lines receiving a high protein diet, thus providing evidence of a significant response to selection of the selected line. No significant differences were found between the selected and control lines remaining on a low protein diet. In this case, selection proved of no great effect. In general, a rather small response to selection was observed. This may be explained by the fact that selection within litters is not highly effective.

The body weights of mice on the 21st, 42nd and 130th day of life were proportional to their weight gains. The lack of differences were: 1. No significant differences between 21 days old in the lines H and Hc. 2. No significant differences between 130 days old in the lines L and Lc, as well as between lines Lc and H × L.

It is of great interest that in the line where females and their offspring were fed with the low protein diet and mated with males from the line main-

Table 1. Mean live weight and weight gains of males in 10th generation

Lines	n	Live weight of 21 days old males $\bar{x}$	n	Live weight of 42 days old males $\bar{x}$	n	Weight gains of males from 21 to 42 days $\bar{x}$	n	Live weight of 130 days old males $\bar{x}$
H	135	8.2 ± 0.13	116	21.2 ± 0.32	116	12.8 ± 0.25	25	29.44 ± 0.48
Hc	89	8.3 ± 0.12	85	18.9 ± 0.33	85	10.6 ± 0.28	24	26.60 ± 0.38
Lc	63	6.3 ± 0.15	54	14.8 ± 0.42	54	8.3 ± 0.31	23	24.62 ± 0.49
L	75	7.6 ± 0.17	69	16.0 ± 0.33	69	8.5 ± 0.27	24	24.11 ± 0.51
H × L	69	5.4 ± 0.14	59	10.9 ± 0.43	59	5.5 ± 0.31	24	23.27 ± 0.65

H - Mice selected on high-protein diet
L - Mice selected on low-protein diet
H × L - Females and progeny selected on a low-protein diet and of males selected on a high-protein diet
Hc - Control mice (nonselected) maintained on a high-protein diet
Lc - Control mice (nonselected) maintained on a low-protein diet
$\bar{x}$ - Mean and standard error
n - number
Highly significant differences were between all lines, exceptions only were:
1. no significant differences between weight gains in the lines L and Lc
2. no significant differences between 21 days old in the lines H and Hc
3. no significant differences between 130 days old in the lines L and Lc, as well as between lines Lc and H × L

Table 2. The activity of aldolase, aminotransferase AspAT and AlAT in liver of 130 days old mice (in units - 1 mg of liver protein)

Lines	n	Aldolase	Aminotransferases	
			AspAT	AlAT
H	25	1.244 ± 0.080	1.642 ± 0.094	0.938 ± 0.046
Hc	24	1.524 ± 0.112	3.175 ± 0.114	1.136 ± 0.061
Lc	23	2.015 ± 0.085	2.587 ± 0.126	0.814 ± 0.058
L	24	1.739 ± 0.074	1.399 ± 0.121	0.845 ± 0.045
H × L	24	1.805 ± 0.059	2.307 ± 0.126	1.288 ± 0.075

H - Mice selected on high-protein diet
L - Mice selected on low-protein diet
H × L - Females and progeny selected on a low-protein diet and males selected on a high-protein diet
Hc - Control mice (nonselected) maintained on a high-protein diet
Lc - Control mice (nonselected) maintained on a low-protein diet
$\bar{x}$ - Mean and standard error
n - number

Significance of differences:

	FDPA	AspAT	AlAT
H and Hc	+	++	+
L and Lc	+	++	-
L and H × L	-	++	++
H and L	++	-	-
Hc and Lc	++	++	++
H × L and Lc	-	-	++

tained on the high-protein diet the weight gains as well as the live weight of 21, 42 and 130 day old males were lowest.

The enzyme activity (Table 2) was differentiated in the five lines and a certain regularity was recorded as regards all three enzymes - i.e., the selected animals, maintained on a high protein diet, clearly demonstrated lower values than did the nonselected mice. An almost identical situation was the case of selected and control animals receiving a low protein diet (aminotransferase AlAT is the only exception here, and then without major significance). The greatest differences in favour of nonselected animals occurred for AspAT: - on a high protein diet 1.533 (3.175 - 1.642) u/mg protein, and on a low protein diet 1.888 (2.587 - 1.399).

4. Discussion

The observations presented should be treated as merely introductory, since they cover - as already indicated - only three enzymes. It seems that it would be premature to conclude that selection, conducted at two levels of protein in food, diminishes, narrows or levels out the variation in enzyme activity. However, the results obtained do seem to be of interest as a model example of biochemical indicators, the behaviour of which was not investigated during selection.

It is also of considerable interest that mice from a grading-up mating were characterised by activity of the two enzymes investigated a little similar to that of the control animals not selected (Lc). The results obtained approach those obtained earlier in experiments on metabolism (Kownacki, Keller and Gebler 1975).

The investigations on the up-grading of animals in different nutritional conditions are similar to the system used in practical animal breeding in several countries. Thus the results of experiments conducted on laboratory animals throw some light on the problem of mass improvement of animals in poor nutritional and environmental conditions, by means of mating with sires from diametrically different - better - conditions. This accords with the opinion of Falconer and Latyszewski (1952), Bateman (1971) and many other authors studying the problem of genotype-environment interaction.

Literature

Badrutdinov, M.G.; Elkin, V.I.: Intensivnost energetičeskogo obmiena u krys raznych linij. Genetika Poviedienija, AN SSSR, Izd. "Nauka", Leningrad, 15-18 (1969)

Baker, R.L.; Chapman, A.B.: Correlated responses to selection for postweaning gain in the rat. Genetics 80, 191-203 (1975)

Bateman, N.: Selection of mice for growth on constant and on changing maize-milk diets. Anim. Prod. 13, 425-440 (1971)

Brown, M.A.; Frahm, R.R.: Changes in postweaning feed efficiency as a result of selection for increased preweaning and postweaning growth rate in mice. Res. Rep. Agric. Exp. Stat. Oklahoma State University, MP-90, 254-259 (1973)

Elkin, V.J.; Fedorov, V.K.: Gibridologičeskoj analiz podviznosti nervnych processov u krys. Genetika Poviedenija, Izd. "Nauka", Leningrad, 81-88 (1969)

Eremiejev, N.S.: Niekotoryje biochimičeskije i morfologičeskije pokazatieli u krys linij i ich gibridov. Genetika Poviedenija, Izd. "Nauka", Leningrad, 88-92 (1969)

Falconer, D.S.; Latyszewski, M.: The environment in relation to selection for size in mice. J. Genet. 51, 67-80 (1952)

Gall, G.A.E.; Medrano, J.F.: Biochemical nature of selection response for increased body size in "Tribolium". 1st World Congress on genetics applied to livestock production, Madrid 1974, Part 3, 541-545 (1974)

Kownacki, M.; Keller, J.; Gebler, E.: Selection of mice for high weight gains. Its effect on the basal metabolic rate. Genetica Polonica 16, No. 3-4, 359-363 (1975)

Krawczyński, J.; Osiński, T.: Laboratoryjne metody diagnostyczne, Państwowy Zaklad Wydawnictw Lekarskich, Warszawa, 373-379 (1967)

Kyle, W.K.; Chapman, A.B.: Experimental check of the effectiveness of selection for a quantitative character. Genetics 38, 421-443 (1953)

Makarova, Z.N.: Izmienienie količestva niekotorych fosforosodierzaščych sojedinienij krovi pri skrieščivanii kur miezporodnom perelivaniukrovi. Avtoref. Kand. Diss. Leningradzkij S-chazjajstviennyj Institut, Leningrad (1967)

Roberts, R.C.: Food consumption in mice selected for high and low body weight. Genetics 74, Nr. 1 Part 2, (Suppl.) 232 (1973)

Roderick, T.H.: Selection for cholinesterase activity in the cerebral cortex of the rat. Genetics 45, 1123-1140 (1960)

Timon, V.M.; Eisen, E.J.; Leatherwood, J.M.: Comparison of ad libitum and restricted feeding of mice selected and unselected for postweaning energetic efficiency. Genetics 65, 145-155 (1970)

Weir, J.A.; Clark, R.D.: Production of high and low blood pH lines of mice by selection and inbreeding. J. Hered. 46, 125-132 (1955)

Received January 30, 1976
Communicated by S. Barbacki

M. Kownacki
Institute of Genetics and Animal Breeding,
Polish Academy of Sciences
Jastrzębiec,
05-551 Mroków (Poland)