Study on the Changes of the Nucleic Acids Content of the Liver and Kidney during Compensatory Growth of the Mice Selected for Resistance to Starvation

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Study on the Changes of the Nucleic Acids Content of the Liver and Kidney during Compensatory Growth of the Mice Selected for Resistance to Starvation

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Summary

In order to compare organ recovery of the two lines of mice selected for high (SH) and low (SL) resistance to starvation and to investigate some aspects of the mechanism of compensatory growth, the weight and nucleic acids of the liver and kidney were investigated during normal and compensatory growth. Male mice of SH and SL lines, at 28 days of age, were randomly allocated to three groups as follows; normal growth group (G-I) and two restricted growth groups which were maintained at a constant body weight during one week (from 28 to 35 days, G-II) and two weeks (from 28 to 42 days, G-III) by restricted feeding and subsequently recovered until 63 days of age by feeding *ad libitum*.

Food restriction resulted in reduction of the total liver RNA, weight/DNA and RNA/DNA and increase of the DNA concentration of the liver. But these parameters of the kidney were not so easily affected as was the liver by food restriction. The SL line was superior to the SH in recovery rate of weight/DNA and RNA/DNA of the liver and the DNA concentration. From the comparison of the nucleic acids of the liver and kidney of both lines between the normal growth group and the two restricted groups, it was suggested that the cells of liver and kidney were affected not only by the time of onset of food restriction but also by its duration.

Wilson and Osborun (1) reviewed the whole subject of compensatory growth, but the physiological mechanism of this response has not been satisfactorily explained.

Recently, cellular aspects of growth in severl organs and tissues were characterized in the rat and other animals (2, 3). Since DNA is constant within a single cell in any species, it is possible to calculate the number of diploid cells in an organ given by analyzing the total organ DNA. Weight/DNA and RNA/DNA ratios of the organ are used as an index of cell size and the DNA concentration is used as a basis of reference to demonstrating changes in cell mass.

Previously, we reported that there were line differences in the recovery rate of body weight and chemical body composition during the compensatory growth of mice selected for high and low resistance to starvation (4).

The object of this research was to compare the organ recovery of these lines of mice and to investigate some aspects of the mechanism of compensatory growth at a cellular level.

Materials and Methods

Experimental animals were derived from two lines of mice selected for high (SH) and low (SL) resistance to starvation. The history of the two selected lines was reported by *Yamagishi* (5). Male mice of both SH and SL lines, at 28 days of age, were randomly divided into a normal group (G-I), one week (G-II) and two weeks (G-III) restricted feeding groups. Total number of the three groups was 30 animals. Mice in the normal group were fed *ad libitum* from 28 to 63 days of age. The body weight of animals in the two restricted feeding groups was controlled by the amount of food offered. The food volume was according to the liveweight recorded daily so as to maintain their body weight for one week (from 28 to 35 days of age, G-II) and two weeks (from 28 to 42 days of age, G-III). After one week and two weeks of restricted feeding, they were fed *ad libitum* up to 63 days of age. Mice were individually reared in wire bottom cages in a temperature controlled-room (24± 1°C) with a 12- hour light cycle and fed powdered food.

Three mice of each group were killed by cervical dislocation on the following days: control group; 28, 35, 42 and 63 days of age, one week restricted group; 35,37 and 63 days of age, two weeks restricted group; 42, 44 and 63 days of age. The liver and kidney were immediately removed, weighted and frozen by storage at -20°C. The entire organs were later thawed and thoroughly homogenized in a glass homogenzier containing ice water. RNA and DNA were separated by modified *Schmidt-Thannhauser-Schneider* procedure recommended by *Munro and Fleck* (6), and individual fractions were assayed for their respective substances. RNA in the hydrolysate was estimated from optical density at 260 nm. DNA in the extract was measured by indole reaction by *Ceriotti* (7). Analyses were carried out in double estimate.

Results

The changes of organ weight, total RNA and DNA, weight/DNA, RNA/DNA and DNA concentration of the liver and kidney from the mice of SH and SL lines are given in Table 1 and 2 and graphed in Fig. 1, 2, 3, and 4.

In the control group, there were no significant differences between the SH and SL lines in weight and total RNA of the liver at any age except at 35 days. But SH line had a greater total liver DNA and less weight/DNA and RNA/DNA of the liver than the SL. On the other hand; weight, total RNA, weight/DNA and RNA/DNA of the kidney of the SL line were greater than those of the SH, and as for DNA concentration, SH line was greater than the SL.

Food restriction resulted in reduction in the total RNA, weight/DNA and RNA/

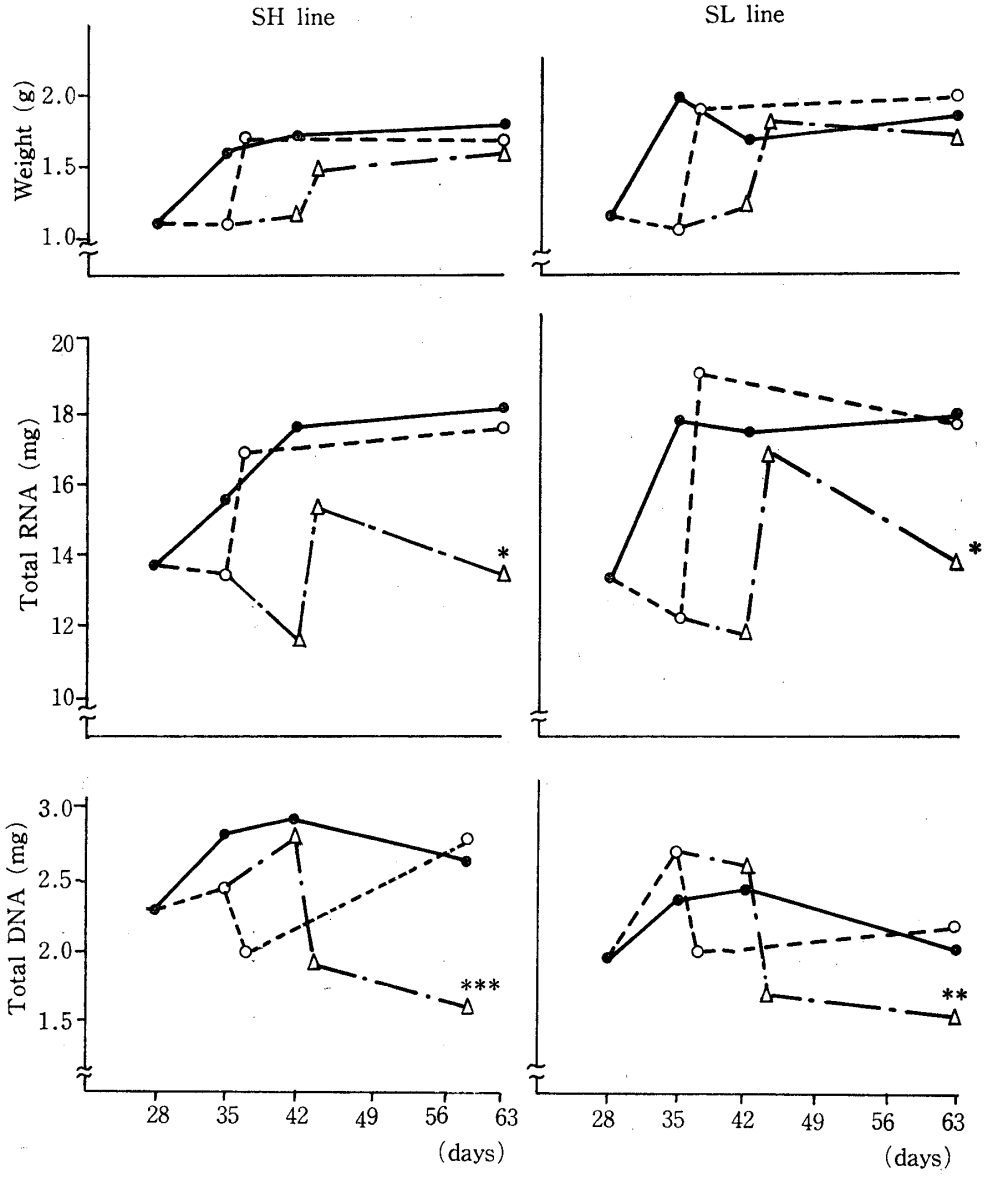

Fig. 1. Changes of weight, total RNA and DNA of the liver during *ad libitum*, restricted feeding and refeeding period.
●—● normal growth group (G-I), o---o one week restricted group (G-II), △—·—△ two weeks restricted group (G-III).
* Significant difference between G-I and G-III at 5% level.
** Significant difference between G-I and G-III at 1% level.
*** Significant difference between G-I and G-III at 0.1% level.

DNA of the liver of both groups (G-II and G-III) and lines, while it didn't reduce these parameters of the kidney in comparison with those at 28 days of age (G-I). At 2 days after refeeding, organ weight, total RNA, weight/DNA and RNA/DNA of the liver and kidney increased rapidly in both lines, whereas total liver DNA and DNA concentration of the liver and kidney decreased.

Table 3 and 4 show the results of an analysis of variance on nucleic acids in the liver and kidney at 63 days of age in both lines. In the liver, there were

TABLE 1. *Total RNA, DNA, Weighht/DNA, RNA/DNA*

Line		Group I			
		28	35	42	63 (days)
Fresh organ weight (g)	SH	1.10[1)]	1.59	1.72	1.78
	SL	1.14	1.97	1.69	1.86
Total RNA (mg)	SH	13.76	15.63	17.74	18.25
	SL	13.44	17.83	17.54	17.96
Total DNA (mg)	SH	2.30	2.80	2.91	2.63**
	SL	1.94	2.35	2.43	2.00
Weight/DNA (g/mg)	SH	0.50	0.59	0.61	0.68
	SL	0.59	0.84**	0.70	0.93
RNA/DNA (mg/mg)	SH	6.01	5.75	6.25	6.97
	SL	6.97*	7.73*	7.24	9.00**
DNA concentration (mg/g)	SH	2.08	1.75	1.68	1.48
	SL	1.71	1.19	1.44	1.08

1) Each figure in the table is the mean of three mice. 2) Significant difference

TABLE 2. *Total RNA, DNA, Weight/DNA, RNA/DNA*

Line		Group I			
		28	35	42	63 (days)
Fresh organ weight (g)	SH	0.26	0.34	0.40	0.37
	SL	0.31	0.51***	0.52	0.53***
Total RNA (mg)	SH	1.51	2.21	2.45	1.99
	SL	2.00*	2.98***	2.77*	2.94***
Total DNA (mg)	SH	0.69	0.97	0.89	0.82
	SL	0.78	0.90	0.96	1.04***
Weight/DNA (g/mg)	SH	0.38	0.35	0.45	0.45
	SL	0.40	0.57	0.54	0.51
RNA/DNA (mg/mg)	SH	2.22	2.27	2.75	2.44
	SL	2.55	3.32***	2.89	2.83*
DNA concentration (mg/g)	SH	2.68	2.90	2.23	2.23
	SL	2.55	1.76	1.88	1.96

1) Each figure in the table is the mean of three mice. 2) Significant difference

line differences in total DNA, weight/DNA, RNA/DNA and DNA concentration except for organ weight and total RNA. Also group differences existed in total RNA and DNA, weight/DNA, RNA/DNA and DNA concentration of the liver. In addition, there was a significant line × group interaction in the liver DNA concentration. In the kidney, line differences in all parameters and also group differences in those except for organ weight and total RNA were observed.

and DNA Concentration in the Liver of SH and SL Lines of Mice

Group II			Group III		
35	37	63 (days)	42	44	63 (days)
1.08	1.69	1.67	1.14	1.49	1.59
1.06	1.89*	1.98*	1.23	1.81*	1.70
13.53	16.96	17.60	11.86	15.45	13.59
12.07	19.18	17.89	11.86	16.93	13.83
2.43	1.98	2.77	2.81	1.91	1.62
2.70	1.99	2.17	2.60	1.69	1.54
0.44	0.86	0.61	0.41	0.79	0.90
0.39	0.95	0.91***	0.48	1.08*	1.11
5.59	8.59	6.46	4.24	8.13	8.35
4.47	9.65*	8.24**	4.60	10.10**	9.01
2.25	1.17	1.65	2.45	1.30	1.04
2.57	1.05	1.10	2.11	0.93	0.91

between SH and SL line. * $P<0.05$ ** $P<0.01$ *** $P<0.001$

and DNA Concentration in the Kidney of SH and SL Lines of Mice

Group II			Group III		
35	37	63 (days)	42	44	63 (days)
0.27	0.38	0.38	0.28	0.35	0.36
0.35**	0.47**	0.54**	0.34***	0.49***	0.51*
1.93	2.50	2.14	1.83	2 14	2.02
2.22*	2.66	2.76**	2.07	2.73***	2.63*
0.77	0.73	0.90	0.68	0.60	0.57
0.89	0.85	0.98	0.80	0.70	0.66
0.36	0.52	0.41	0.42	0.59	0.63
0.39	0.55	0.55	0.43	0.70	0.77
2.53	3.42	2.36	2.74	3.55	3.55
2.49	3.12	2.83	2.60	3.89	3.99
2.81	1.92	2.42***	2.42	1.71	1.60
2.58	1.83	1.82	2.32	1.43	1.30

between SH and SL line. * $P<0.05$ ** $P<0.01$ *** $P<0.001$

Discussion

i) Comparison of organ recovery of the two lines of mice at cellular level.

In the control group, from the comparison of total DNA, weight/DNA, RNA/DNA and DNA concentration of the liver, it was suggested that the SH line had a larger cell number, smaller cell size and larger cell mass than the SL. Furthermore, it was noticed that kidney weight, cell size and cell mass of the SH line were

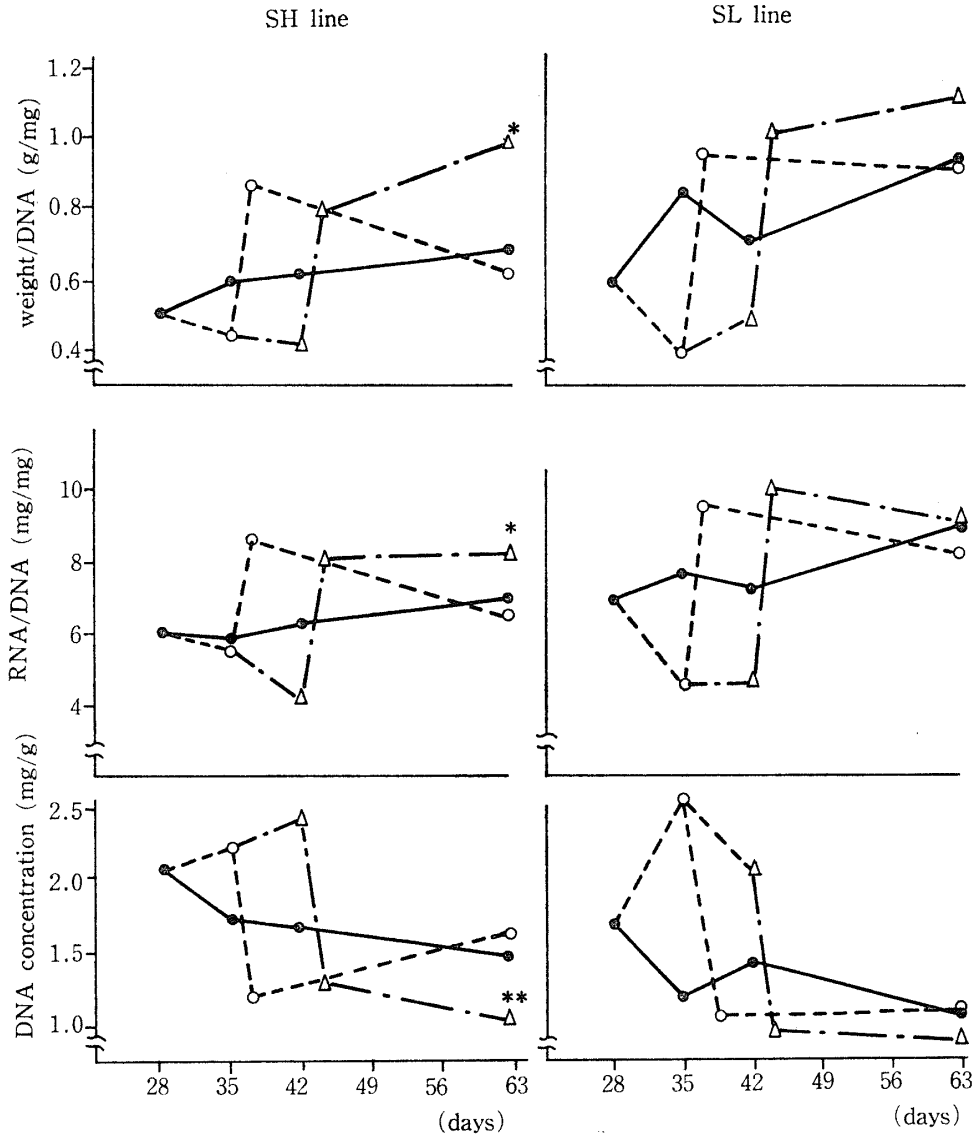

Fig. 2. Changes of weight/DNA, RNA/DNA and DNA concentration of the liver during *ad libitum*, restricted feeding and refeeding period. ●—● normal growth group (G-I), o---o one week restricted group (G-II), △---△ two weeks restricted group (G-III). * Significant difference between G-I and G-III at 5% level.

TABLE 3. *Analysis of Variance on Nucleic Acids Content of the Liver at 63 Days of Age*

Source	df	Mean squares for:					
		Fresh organ weight	Total RNA	Total DNA	Weight/ DNA	RNA/DNA	DNA concentration
Line	1	0.12	0.03	0.85**	0.23**	9.95**	0.58**
Group	2	0.06	33.73**	1.36**	0.14**	2.26**	0.26**
Line × Group	2	0.02	0.16	0.14	0.01	0 80	0.07*
Error	12	0.04	2.32	0.06	0.01	0.38	0.02

* $P<0.05$ ** $P<0.01$ Significant difference.

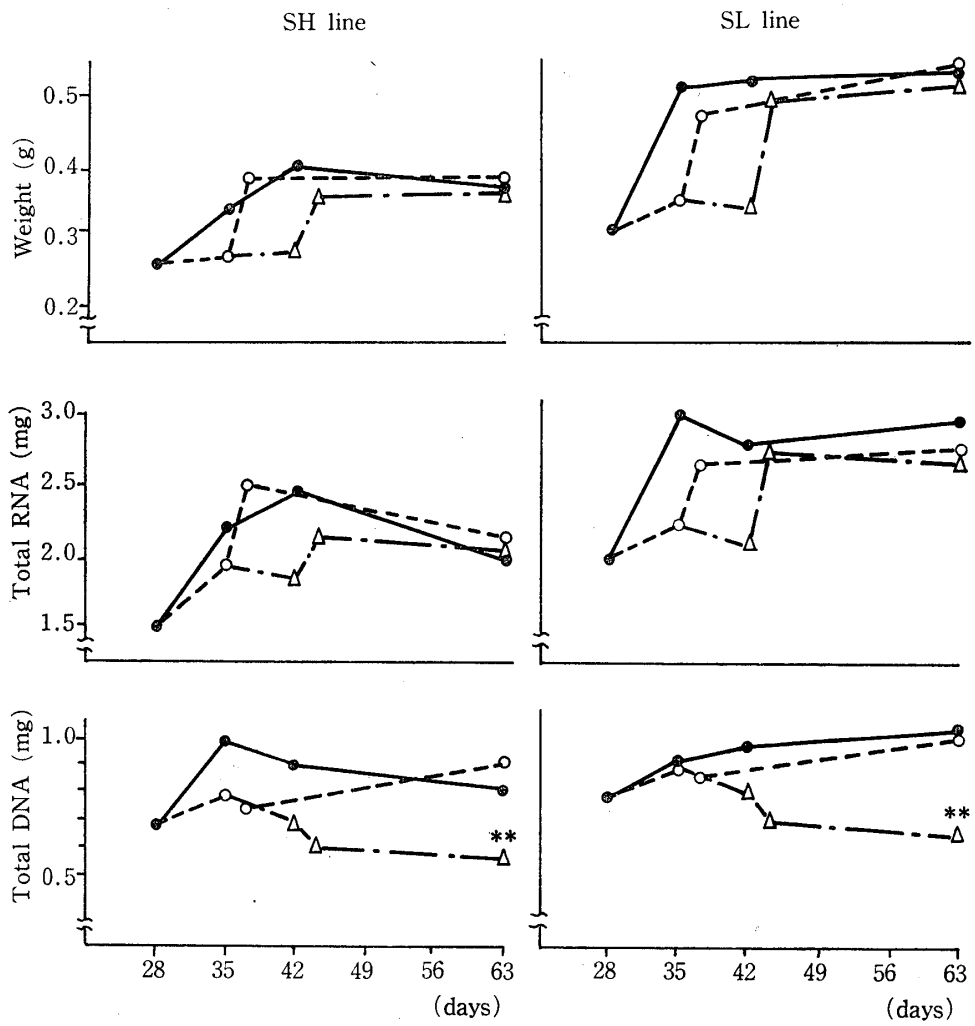

Fig. 3. Changes of weight, total RNA and DNA of the kidney during *ad libitum*, restricted feeding and refeeding period. ●—● normal growth group (G-I), ○---○ one week restricted group (G-II), △–·–△ two week restricted group (G-III).
** Significant difference between G-I and G-III at 1% level.

TABLE 4. *Analysis of Variance on Nuclei Acids Content of the Kidney at 63 Days of Age*

Source	df	Mean squares for;					
		Fresh organ weight	Total RNA	Total DNA	Weight/ DNA	RNA/DNA	DNA concentration
Line	1	0.117**	2.38**	0.075**	0.060**	0.849**	0.68**
Group	2	0.001	0.04	0.202**	0.096**	2.655**	0.86**
Line×Group	2	0.0001	0.06	0.009	0.003	0.002	0.05
Error	12	0.002	0.04	0.006	0.002	0.035	0.01

** $P<0.01$ Significant difference.

smaller than those of SL and the SH line had a smaller cell number in the kidney than did the SL. The SH line had more accumulated body fat than the SL (4) and, in general, it is known that liver is important organ for lipid synthesis. Further

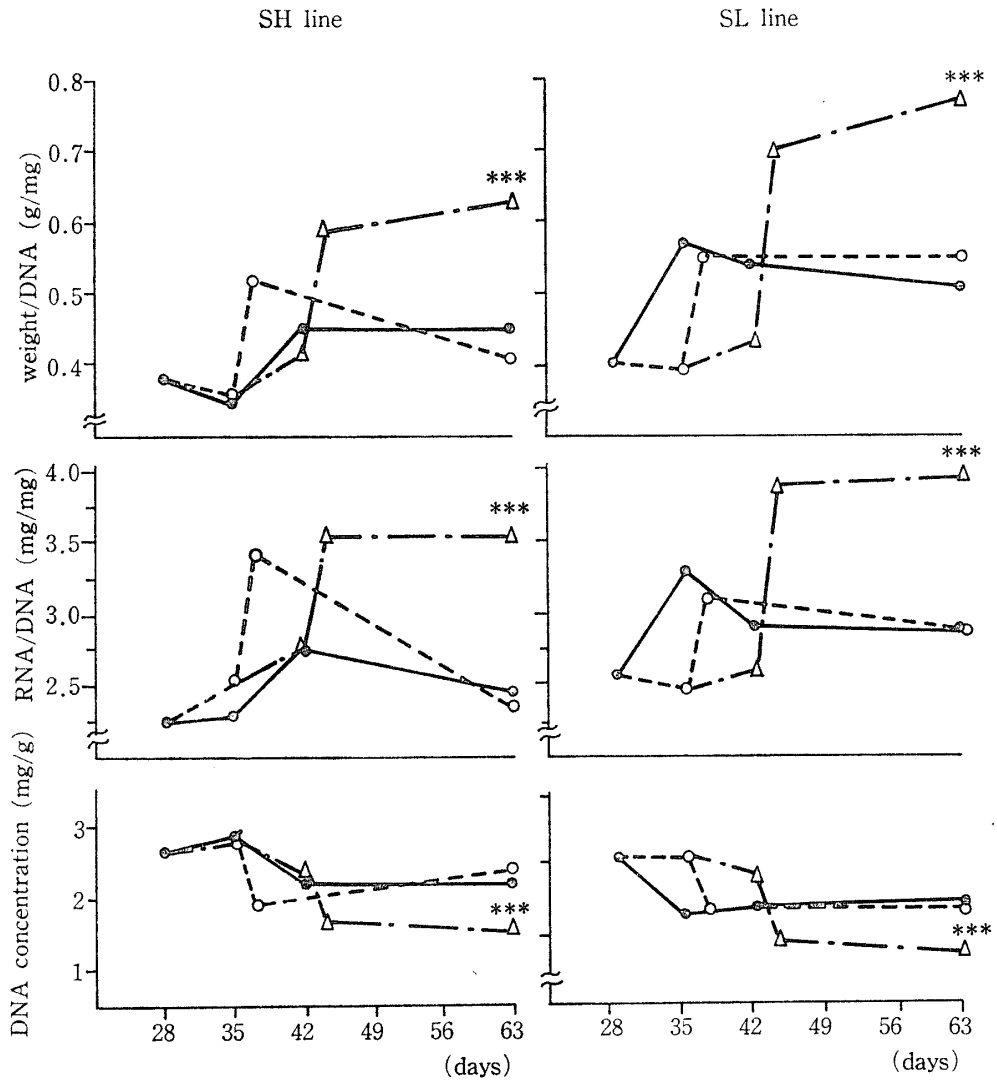

Fig. 4. Changes of weight/DNA, RNA/DNA and DNA concentration of the kidney during *ad libitum*, restricted feeding and refeeding period. ●—● normal growth group (G-I), ○---○ one week restricted group (G-II), △---△ two weeks restricted group (G-III). *** Significant difference between G-I and G-III at 0.1% level.

studies would be necessary to provide information on how the differences of liver cellularity and lipid synthesis between SH and SL line are related. And also, it is necessary to clearily how the function of the kidney relates to the resistance to starvation.

The effects of food restriction on the liver cell size and cell mass observed in the present investigation were in agreement with previous reports on the liver tissue (8) and also on the skeletal muscle (9, 10). But in our experiment, kidney cell size and cell mass of both lines were not reduced by food restriction. These findings suggested that the kidney was not so easily affected as the liver by food restriction.

At 63 days of age, there are no signfiicant differences in the liver cell size and cell mass between the control and two weeks restricted group of the SL line, although

there are signfiicant differences between those groups of SH. This result suggests that there are line differences in the recovery of cell size and cell mass from food restriction. In the previous report, we suggested that the mice of the SL line were superior to SH in recovery of body weight and chemical body composition during compensatory growth (4). In the present experiment, it was also cleared that the SL line was superior to SH in the recovery rate of the cells of the liver.

ii) The mechanism of compensatory growth at cellular levels.

At 2-days after recovery, total RNA, weight/DNA and RNA/DNA of the liver and kidney increased rapidly, but total DNA and DNA concentration of these decreased in both lines. These results may suggest that, in the early period of recovery, the synthesis of RNA and other cellular constitutents are conducted more rapidly than the DNA synthesis.

At 63 days of age, the two weeks restricted group could not recover all parameters except weight/DNA, RNA /DNA and DNA concentration of the liver of SL line and total RNA of the kidney of both lines in comparison with the normal group. *Winick and Noble* (2) hypothesized that normal growth was divided into 3 periods: cell division alone; cell division with concomintant cell enlargement; and cell enlargement alone with no futher increase in number of cells. Thus they suggest that cellular effect of malnutrition depends on the phase of growth in the animal at the time of malnutrition (8). In our experiment, the age of onset of food restriction, 28 days of age, may be the stage of cell division with concomitant cell enlargement. Also present results clearly demonstrate that cellular aspects of liver and kidney were affected not only by the time of onset of food restriction but also the duration of food restriction.

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