CALCIUM METABOLISM IN GROWTH OF TWO STRAINS OF MICE AND THEIR RECIPROCAL HYBRIDS

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Fenton *et al.* (1–4) have suggested that the nutritive requirements, particularly in various kinds of protein, fat and vitamins in mice differ with each strain. Kondo *et al.* (5) have reported that there are significant differences in digestibility of dry matter and crude protein among the four strains of mice. Rutman (6) observed that there are some differences in the rate of protein synthesis between the high growth rat, F strain and J strain which have low growth.

Numerous studies have been reported on the calcium metabolism of the experimental animals and farm animals, but there are only few studies on the mice, and especially little is known concerning the differences of calcium metabolism in each inbred strain of mice which have differentiated genetical characteristics and between inbred and hybrid mice, and the changes of calcium metabolism in the various stages of growth.

In this report, the comparisons of the calcium metabolism in the 40, 50 and 60 days old male mice of two inbred strains, DD and RR, and their reciprocal hybrids, DR and RD were evaluated on the retention of Ca^{45} in femurs and excretion of radiocalcium after the intraperitoneal injection of radioactive calcium.

Materials and Methods

Two strains of inbred mice, DD and RR, and their reciprocal hybrids(F_1), DR and RD, used in this experiment were produced in our faculty. They were fed on the stock ration used in our laboratory. The percentage composition of this ration are as follows: polished barley 40, ground corn 25, wheat bran 20, fish meal 15, NaCl 0.5 and $CaCO_3$ 0.5 percent, and it contains approximately 19 percent of crude protein, 0.92 percent of calcium and 0.90 percent of phosphorus.

Each of the five male mice of the four different lines, two inbreds and two hybrids, at approximately 40, 50 and 60 days of age were selected from each litter of the mice which were born nearly on the same day at each experimental day. The mice were starved for 16 hours to be sure that they had a fairly uniform quantity of feed in the digestive tract at the time Ca^{45} was administered. They were then allowed access to feed for one hour after which the feed was again removed. One hour after the feed was removed, the mouse was administered $2\mu c$ of $Ca^{45}Cl_2$ (0.03 mg Ca/0.1 *ml* neutral solution) by the intraperitoneal injection. Following administration of the isotope, five mice of each line were placed in wire metabolism cages to collect their excreta and were fed the diet as usual. The mice were sacrificed by ether at 24 hours after administration of radiocalcium. After the mice were sacrificed both femurs (including kneepan) were taken and dried, and then dry ashed in an electric furnace at 600°C. The metabolism cages were washed with distilled water sufficiently, then feces, urine, the scattered diet and washings were taken into the evaporating dish, and after evaporation and ashing, the radioactivity was determined as total excretion of radiocalcium during 24 hours period. Total and radioactive calcium values were determined from the oxalate precipitate, as described by Comar *et al.* (7).

Results and Discussion

1. Body weight and the composition of femur

The age of the mice sacrificed, body weight and chemical analyses (dry matter, ash and calcium contents) of the femur in mice of four lines are shown in Table 1.

Body weight at the 40th day of three lines except RR mice were approximately of the same value. After then, hybrid mice, DR and RD showed the intermediate body weight of the two parental strains of inbred mice, DD strain which had a rapid growth response and heavy body weight and RR had a slow and light one, generally.

The increasing tendency of dry weight, ash and calcium contents of the femur at each experimental day of three times in growth stage of four lines of mice have much in common with the body weight of each line. The increasing rates of them in DD mice from the 50th day to 60th day are very low. RR mice show the lowest value in the composition of the femur in all lines. The chemical composition of the femur of the hybrid mice was between the inbred mice DD and RR, in most cases, but that of the 60 days old DR mice were higher than of the inbred DD mice. It seems, therefore, that the hybrid DR mice have shown the heterosis on body weight and the composition of the femur.

Kondo *et al.* (5) have reported that the feed consumption which was

calculated by the chromic oxide indicator method, of DD and RR strains which had received the same diet in this experiment are 280 mg and 300 mg per gram of body weight daily, respectively. On the basis of these data, the daily

Table 1. Body weight, chemical analyses of femurs and radioactivities in femurs and excreta of four lines of mice at each experimental day.

Line of mice	Exp. No.	Age	Body weight	Femur dry matter	Femur Ash	Femur Ca	D.M./B.W.	Ash/D.M.	Ca/Ash	% of dose of Ca^{45}	
										Femur	Excreta
		(day)	(g)	(mg)	(mg)	(mg)	(mg)	(%)	(%)		
DD	1	40	16.4±0.9*	43.6±3.1	24.0±2.0	9.7±0.7	2.7	55.0	40.3	7.0±0.5	9.6
	2	51	20.7±1.0	58.4±1.8	35.2±1.0	13.8±0.5	2.8	60.4	39.1	6.6±0.4	12.0
	3	61	22.8±1.1	60.3±4.1	37.0±3.1	14.4±1.0	2.6	61.4	38.9	6.3±0.2	25.4
RR	1	41	14.4±1.1	32.9±4.0	17.0±2.2	6.8±0.9	2.3	51.6	39.8	7.5±0.3	19.0
	2	51	17.5±0.7	44.7±1.2	26.1±0.7	10.5±0.6	2.6	58.4	40.2	6.8±0.8	9.1
	3	63	19.6±0.3	53.8±5.7	33.3±4.0	13.0±1.3	2.7	61.9	39.1	4.6±0.2	15.2
DR	1	40	16.7±0.9	39.6±1.0	22.1±0.8	8.8±0.3	2.4	55.9	39.7	7.3±0.7	7.5
	2	51	19.9±0.7	54.3±1.4	33.1±2.6	12.8±1.0	2.7	61.0	38.7	6.6±0.6	12.7
	3	63	23.3±1.0	66.3±2.2	40.4±1.6	15.8±0.6	2.8	60.9	39.0	4.7±0.2	11.6
RD	1	39	16.9±0.2	40.1±1.3	22.3±0.7	8.8±0.4	2.4	55.5	39.4	7.4±0.3	8.2
	2	51	18.2±0.8	50.5±2.3	29.5±1.4	11.6±0.5	2.8	58.4	39.3	6.9±1.0	10.3
	3	65	21.0±0.5	57.1±2.5	35.0±2.0	13.7±0.7	2.7	61.3	39.2	6.1±0.4	5.9

* Mean ± Standard deviation (five mice)

feed and calcium consumption by DD and RR strains during the first period of ten days (from the 40th to 50th day) were calculated from the average of body weight of the 40 and 50 days old mice, and the consumption in the second period were obtained from the average body weight of the 50 and 60 days old animals.

Table 2. Daily calcium consumption and the increase of femur calcium in DD and RR strains of mice at two periods of ten days.

Strain of mice		DD		RR	
Period	(age in day)	40-50	50-60	40-50	50-60
Average body weight	(g)	18.6	21.8	16.0	18.6
Feed consumption	(mg/day)	5194	6090	4758	5565
Calcium consumption	(mg/day), A.	47.8	56.0	44.0	51.2
Increase of femur Ca	(mg/day), B.	0.38	0.06	0.37	0.21
B/A	(%)	0.78	0.10	0.85	0.42

Table 2 shows these data and the average increasing rate of femur calcium per day for the daily calcium consumption during each two period of ten days.

In DD mice, 0.78 percent of the daily calcium consumption are retained in the femur for the former period of ten days, and a less amount (0.10 percent) of the dietary calcium are deposited in the femur at the next ten days. On the other hand, RR mice in the later ten days period have deposited a

half value of the former period. It seems, therefore, that DD mice have almost completed the bone formation at about 50 days old.

The femur dry matter per gram of body weight, bone ash percent and calcium percent of bone ash in all lines of mice at each age are presented in Table 1. The femur dry matter per gram of body weight of the 40 days old DD mice showed a value higher than that of the other three lines and increased to the 50th day slightly and at the 60th day it was decreased, thus it seemed that the bones of DD strain were completed at 50 days old as described above. The femur dry matter in three lines of mice except of DD, increased during the period from 40 days of age to 50 days sufficiently and a less increase occurred in the later period of ten days, comparatively. The increasing rate during the later period of ten days in RR and DR were equal to that of the former period of DD mice.

The bone ash percent on the dry basis of the 40 days old RD mice are significantly lower than that of the other three lines. These results have suggested that the bones of the 40 days old RR mice are under the growing stage and have a high amount of the organic substances. The increase of the bone ash percent of RR mice in the former period of ten days continued to the next period, then it appears that the active deposition of minerals in the femur succeeded all over the experimental periods. Ash percent of the femur in DD and DR mice were increased in similar rate during the first period of ten days and showed nearly identical values at 50 and 60 days old. All mice at about the 60th day showed approximately the same ash percent of the femur, 61 percent and it was in agreement with the value of C3H strain mice reported by Nerurkar and Sahasrabudhe (8).

The calcium percent of bone ash in DD and DR mice were decreased from the 40th to 50th day and with no changes thereafter. As there were no significant changes on the amount of calcium deposited by the bones after the dietary calcium was absorbed from the intestinal tract during the latter period of ten days, the deposition of minerals including calcium by bones had maintained the same rate, and it seemed, therefore, that the bone grew under a constant rate in each constituent. In the 40 days old RR mice, femur ash percent was lower than the other three lines of mice, but calcium percent in the ash was as in the other lines, and it increased in the former period of ten days and decreased in the latter period. These facts suggest that the absolute quantity of calcium in the bone of RR mice increase in these periods and the deposition of the other minerals in the bones have exceeded the uptake of calcium. The calcium content of the femur in RD mice was observed to be of the same percentage at each experimental day.

From the results and discussion described above, it was supposed that the mode of the deposition of bone forming substances until the completion of

bone growth should be as follows. The more deposition of inorganic substances including calcium occurs in the bone which are comparatively rich in organic matter, and then the retention of calcium increases until the calcification is completed.

2. *Radioactivity in femur*

The radioactivity in the femur of the four lines of mice at each three times of growth stage are presented in Table 1 and to clarify the relations between the dietary calcium and the radioactive calcium on the deposition in the femur and the transfer in body, calcium content, percent of dose of Ca^{45} and its specific activity of the femur are shown in Figures 1, 2 and 3 by graphical representation.

The retained radioactivities in the femur of the four lines of mice for 24 hours after the intraperitoneal injection of the isotope occupied 7.0–7.5 percent of the dose at the 40th day and 6.5–7.0 percent at the 50th day. Percent of dose of Ca^{45} in the femur of the 40 days old mice are in agreement with the value of rat by Lengemann and Dobbins (9) which indicate that 7.6 percent of the intraperitoneally injected radiocalcium deposit in the femur of female young rat which have received a control diet for 24 hours after the administration of the isotope. It is presumed that essentially no differences occur on normal calcium metabolism between mouse and rat.

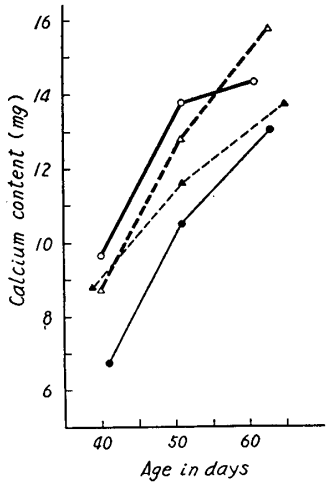

Fig. 1. Calcium content of femur.

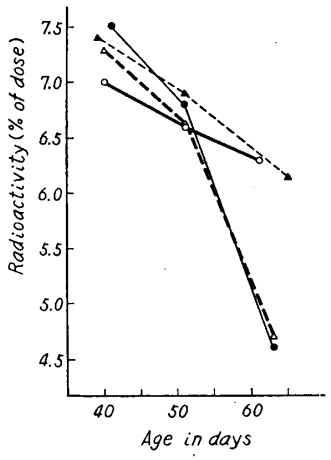

Fig. 2. Radioactivity in femur.

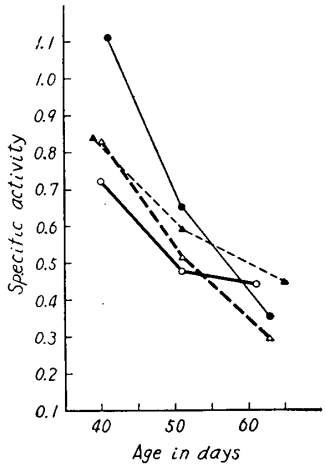

Fig. 3. Specific activity of Ca^{45} in femur.

Specific activity = $\frac{\%\text{ of dose of } Ca^{45}}{\text{Total Ca mg}}$

DD ○——○ RR ●——● DR △- - - -△ RD ▲- - - -▲

Two decreasing tendencies were observed on the radiocalcium of the femur in mice of the four lines during the latter period of ten days. One of them, the retention of Ca^{45} in the femur of DD and RD mice slightly decreased

after the 50th day continuing the decrease of the first period of ten days, therefore the deposition of Ca^{45} in the femur decreased from the 40th to the 60th day at a constant rate. The other one was observed in RR and DR mice. The decreases in the first period of ten days were the same as in the one of DD and RD mice, and at the 60th day, the deposition of Ca^{45} in the femur showed sufficient lower contents, approximately 4.6 percent of the dose, in RR and DR mice compared with the other two lines.

The specific activity (Ca^{45} percent of dose/Total Ca mg) of the femur in RR mice was higher than the other three lines at the 40th day. Afterward, these values of all lines of mice were decreased according to the increases of body weight. The decreasing rates of the specific activity were divided into two groups, one was observed in DD and RD mice, and the other one in RR and DR, and the former group decreased slowly and the latter sharply. As the specific activity of the femur in DD strain mice decreased sharply from the 40th to 50th day and at 60 days of age showed nearly indentical values of the 50th day, it seemed that the utilization of the dietary calcium by the femur were increased at the first period of ten days, and thereafter no changes occurred in the utilization of calcium in feed.

The deposition of Ca^{45} in the femur of the 40 days old RR mice after intraperitoneal injection of radiocalcium are nearly identical with that of the other three lines but as the specific activity in this strain are higher than the other three, it is supposed that the utilization of the dietary calcium by RR mice are lower than that of the other mice at the early growth stage. After 50 days of age, the higher specific activity of the femur in RR mice was not observed as compared to the other mice. Therefore, the deposition in the femur of Ca^{45} which was administered with the intraperitoneal injection decreased and the utilization of the dietary calcium increased. The 60 days old RR mice retained a significant lower percentage of the dose of Ca^{45} in their femur and contained a lower calcium percent in bone ash and a higher total calcium content in the femur than the 50 days old mice, as previously mentioned. Therefore, the deposition of the dietary calcium and intraperitoneal dosed calcium by bones are very little and the metabolic rate of calcium in bone are small, and it is supposed that the turnover rate of calcium in the bone are small and the dietary calcium are surely depositing in the femur of RR mice at about the 60th day. These facts are so with the hybrid DR mice.

As a thing in common of calcium metabolism in the four lines of mice, it was observed that when the more increase of body weight at each growth period occurred, the more dietary calcium was retained in the bone compared to the rate of retention of radiocalcium which was administered with intraperitoneal injection, and it was supposed that the transfer of calcium in

the body had occurred in comparatively low rate. However, in DD and RD mice if the changes of the radioactivity in the excreta are left out of consideration, it appears that the more excretion of calcium occurs in disregard of these mice might be expect to absorb the more dietary calcium from the gut.

3. Radioactivity of excreta

The results of radioactivity measurements of excreta collected 24 hours after administration of the isotope are presented in Table 1.

In comparison of the excretion of Ca^{45} in the excreta of four lines of mice at each age, the 40 days old RR mice excreted two times of the other three lines, and at the 50th day, the excretion of each line showed a similar percent of dose of Ca^{45}, and at the 60th day, each group varied in their excretion and DD excreted about four times of RD mice.

The mice of DD strain showed the same excretion of Ca^{45} at the 40th and 50th day and two times of percent of the dose in the excreta of the 50 days old mice was excreted at the 60th day of this strain through the bloodstream from intra-peritoneum. On the other hand, as previously mentioned, the retention of Ca^{45} in the femur of the 60 days old mice was the same as that of the 50 days old and furthermore the utilization of the dietary calcium was shown in good degree and less calcium was retained in the bones from the diet. It appears, therefore, that the functions of absorption and excretion of calcium in the intestinal tract may be sufficiently high although the apparent utilization of the dietary calcium is decreased.

The deposition of radiocalcium in the femur of RD mice showed a value similar to the DD mice and there were no significant differences between the 50 days old mice and 60 days old, but at the 60th day, RD mice had excreted only one fourth of the radiocalcium in the inbred DD strain and the increase of calcium content of the femur during the second period of ten days had been continued from the first ten days period, it is considered, therefore, that the absorption of the dietary calcium should be comparatively high. It appears that the mode of calcium metabolism from 50 days of age to 60 days in the inbred DD mice are essentially different from the hybrid RD mice, although the physiological states of the bone in both mice are similar at the 60th day as previously described.

The 40 days old RR mice excreted 19 percent of the dose of Ca^{45} after the intraperitoneal injection of the isotope, and it was about two times of the excretion of the other three lines, and then the retention of calcium in the body of RR mice was lower than the other mice and the specific activity of the femur was higher in RR strain owing to the low calcium content in the femur although the radioactivity in the femur showed values similar to the other three lines of mice.

Then it is supposed that the 40 days old RR mice have shown the low absorption of calcium not only apparently but also true absorption from the intestinal tract substantially. At the 50th day, RR mice excreted only a half of the radiocalcium in the excreta of the 40 days old mice and the retention of Ca^{45} was slightly lower than the 40 days old. It appears, therefore, that the metabolic calcium level of RR mice is trending toward an increase after the 40th day of age. At the 60th day, the excretion of Ca^{45} increased slightly and the retention decreased sufficiently and furthermore the calcium content in the femur increased as described above, and it was supposed that RR mice had continued the high metabolic calcium level by the dietary calcium even after 50 days old.

There were not many changes in the excretion of radiocalcium of the hybrid mice, DR and RD at each experimental day and it was stable as compared with the inbred mice, DD and RR.

Conclusions

DD inbred mice: The bone of the inbred strain DD mice until about 40 days old have grown actively and during the first period of ten days the cessations of body weight gain and the calcification of bone have occurred and at about the 50th day the mineralization of the bone should be complete sufficiently, afterward, the growth and the retention of calcium in the bone should enter into the inactive period. However, it seems that the transfer of calcium in the digestive tract are further active during the second period of ten days although the apparent utilization of the dietary calcium decrease after the increased excretory rate of calcium from the body store.

RR inbred mice: The utilization of the dietary and stored calcium of the 40 days old RR mice are not sufficient and increase toward the 50th day gradually and afterward a large amount of calcium is transferred for the bone formation. At 60 days old, the retention of calcium in the femur are slower than that of the other minerals and an increase of excretion occurred. It appears that after the 60th day, RR mice may enter the cessation period of bone growth. In general, the growth, bone formation and calcium metabolism of RR mice are 10 to 15 days behind for DD strain.

DR hybrid mice: The utilization of the dietary calcium by the hybrid DR mice are not sufficient during the first period of ten days and thereafter the dietary calcium are utilized actively for the bone formation, although the transfer of calcium in the body are lower than the inbred mice. Therefore, DR mice had shown similar states on the bone formation and calcium metabolism of RR mice, although DR mice showed the heterosis partially in their body weight and the compositions of bone.

RD hybrid mice: The variation of calcium metabolism in growth process

of the hybrid RD mice are smaller than that of the other lines of mice, generally. The utilization of the dietary calcium by this mice at about the 50th day are not sufficient to extend the increase of body weight gain. In RD mice, it appears that the absorption of the dietary calcium and the retention of calcium which was once absorbed from the gut during the second period of ten days may substantially increase. Then that the active metabolism of calcium in RD mice have occurred in the latter period is in accord with the inbred DD mice, however, it appears that their metabolic functions on the availability of the dietary calcium are contrary to each other fundamentally.

Summary

The comparisons of the calcium metabolism of the 40, 50 and 60 days old male mice of two inbred strains, DD and RR, and their reciprocal hybrids (F_1), DR and RD were evaluated on the chemical composition of femurs and the retention and excretion of radiocalcium after the intraperitoneal injection of the isotope.

1. Dry weight of femurs per gram of body weight of the four lines of mice at each growing period varied from 2.3 to 2.8 mg, and the bone ash percent of all mice except the 40 days old RR strain and calcium percent of femur ash of all mice varied from 55 to 62 percent and 38.7 to 40.3 percent, respectively. In femurs of all groups with the exception of the 60 days old RR and DR mice, 6.0 to 7.5 percent of the dose of Ca^{45} were retained for 24 hours after the administration of radiocalcium intraperitoneally. The excretion of radiocalcium of each mice varied with very wide ranges, and the minimum excretion was 6 percent of the 65 days old RD mice and the maximum was 25 percent of DD mice at 61 days old.

2. There were significant differences in the growth and calcium metabolism between the inbred mice DD and RR. DD mice almost completed the bone formation at about 50 days of age and thereafter the utilization of the dietary calcium decreased gradually. On the other hand, at 50 days old, RR mice were beginning to utilize the dietary calcium actively for the completion of bone formation.

3. The hybrid mice, DR showed insufficient utilization of the dietary calcium at about 40 days of age, and after the 50th day they retained a large amount of calcium for the formation of bones. However, it seems that the transfer of calcium in body of the 50 days old mice are slower than that of the more early growth stages, and the type of metabolic process of calcium in this mice resemble the RR inbred mice.

4. The other hybrid mice, RD showed less varieties of calcium metabolism at each experimental time compared to the other lines generally. After

50 days old, the absorption of the dietary calcium and its metabolism in the body increased actively as shown in DD strain, however, the fundamental differences in the actual utilities of calcium are recognized between RD and DD mice, and the hybrid RD mice have had the superiority on the essential utilization of calcium over the inbred DD mice.

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