CLINICAL STUDIES ON SERUM IONIC CALCIUM IN DOMESTIC ANIMALS

X. EFFECT OF ORAL ADMINISTRATION OF CALCIUM ION SOLUTION ON RATS FED A SEVERELY LOW CALCIUM DIET

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A large portion of the calcium in the body exists in the bone. The significant function of the body is due to the action of the ionic calcium which is physiologically active. It is well known that the ionic calcium controls the stimulation of the joint part in muscle and nerve, and that it decreases the permeabilities of a capillary blood vessel and a cell-membrane\(^2\). Moreover, it is a need factor for muscular contraction, transmission of nervous agitation, and coagulation. The development of the bone in growing animals is especially excellent, as the amount of calcium occupies approximately 50 per cent of the bone constituents. Therefore, it is easy to understand that a growing animal needs much calcium.

Although the metabolic mechanism of calcium in the body is exceedingly complex, healthy animals have an adaptation ability to the deficiency of calcium intake with the help of reserve calcium and bone calcium in the body. They keep the calcium level in the serum within the limit of the normal range in general\(^5\). The body has such a physiological function that calcium absorption from the intestinal canal is controled, when too much calcium is present in the body, and that it is stimulated when too little calcium remains in the body\(^6,7\). It has been known from an experiment on calcium absorption that the increase of calcium in the blood is caused by the increase of ionic calcium alone\(^9\), and that the absorption of phosphoric acid decreases when the ratio of Ca/P is high, since this acid is absorbed after the absorption of calcium in the diet\(^6,7\). Furthermore, it is presumed that there are many differences in the amount of calcium which animals take in general. As long as the authors know, however, no report has been made as yet on the influence of the over intake of calcium upon growing animals fed the standard calcium diet with an addition of a calcium ion solution (CS). In the present series of studies, the authors have noticed experimentally and clinically a decreasing tendency of serum ionic calcium (SIC) in dogs and rabbits with the liver damaged by such diseases as hepatitis, heartworm disease, and hookworm disease. It was already reported that the last named of the disease caused anemia as a main symptom\(^14\). On the other hand, in healthy growing rats which had been fed a severely low calcium diet, it is a general tendency to observe a decrease of SIC, a hypofunction of hematopoiesis, and a slight dysfunction of the liver accompanied by metabolic abnormalities in the body\(^15\). Moreover, it was proved that rats maintained on a severely low calcium diet were infected with *Ancylostoma caninum* given perorally as an external force to the whole body. Then the authors believed that the capacity of body defence reaction and the resistance to

infection decreased clearly in the body of such animals\textsuperscript{22}.

The present study was carried out to determine as influence of a standard calcium diet with an addition of CS given perorally on the body in hypo-ionic calcemia caused by feeding of a severely low calcium diet, and also to clarify the significance of the oral administration of CS in healthy growing rats. In consequence, the authors obtained interesting results, which are presented in this paper.

MATERIALS AND METHODS

1. Experimental animals

Twenty-seven male rats, 5 weeks old, weighing approximately 90 g, were selected. They had been fed a standard rat diet (manufactured by the Oriental Co., Tokyo) for a week until they were used for experiment. At the end of the week they were divided into three groups and placed on experimental diets. The first group of 6 rats was fed the standard calcium diet with an addition of CS (a high calcium diet), the second group of 9 rats the standard calcium diet, and the third group of 12 rats a severely low calcium diet.

2. Experimental diets

The compositions of the experimental diets used were mentioned in the previous paper by the authors\textsuperscript{21}. Analyses of the diets showed that the standard calcium diet contained 0.414% calcium and the severely low calcium diet 0.002% calcium per 100 g of the respective experimental diet. The CS used was a solution which contained only 75 mg of Ca\textsuperscript{++} per 100 ml.

3. Experimental methods

The three groups of rats were all placed on the experimental diets, as shown in Chart 1. The standard calcium diet was given to 9 rats (RSCD), the high calcium diet to 6 rats (RSCD+Ca\textsuperscript{++}), and the severely low calcium diet to 12 rats (RLCD). Three of the 9 rats of group RSCD were fed a high calcium diet (RSCD+Ca\textsuperscript{++}) for a period of 2 weeks from 3 to 5 weeks after the standard calcium diet had been given (a.d.). The rats of group RLCD were further divided into three subgroups of 3 rats each. Three subgroups of the four were fed the standard calcium diet (RLCSD), the high calcium diet (RLCSD+Ca\textsuperscript{++}), and RLCD, respectively, from 3 to 5 weeks a.d. In consequence, all the rats were placed on 6 different diets, RSCCD+Ca\textsuperscript{++}, RSCD+Ca\textsuperscript{++}, RSCD, RLCSD+Ca\textsuperscript{++}, and RLCD, for a period of 2 weeks from 3 to 5 weeks a.d.

The rats had all been given one of the experimental diets and tap water freely until they were sacrificed for autopsy. Some of them were made to drink supplementary CS, instead of tap water. The methods used for the analysis of blood, tissue, and body constituents were the same as mentioned previously by the authors\textsuperscript{24-22}.

RESULTS

1. Variations of body weight in the groups (Chart 2)

The measurements of body weight are shown as the averages of the total numbers of experimental rats in each group until 3 weeks a.d. and as the average of three rats in
every group after that.

The average body weight was 105 g in all the rats before feeding of the experimental diets (b.d.). It showed a tendency to increase gradually in group RSCCD+Ca++, in which it was 130, 149, 167, 178, and 192 g 1, 2, 3, 4, and 5 weeks a.d., respectively.

The average body weights were 118, 137, 151, 163, and 175 g in group RSCD 1, 2, 3, 4, and 5 weeks a.d., respectively, and 166 and 184 g in group RSCD+Ca++ 4 and 5 weeks, respectively. The increasing tendency of body weight was more remarkable in group RSCD+Ca++ than in group RSCD, but there was no significant difference in either value at 4 and 5 weeks a.d. between the two groups.

In group RLCD, the body weight showed a tendency to decrease gradually, being 104, 91, 75, 66, and 62 g 1, 2, 3, 4, and 5 weeks a.d., respectively. In groups RLCSD+Ca++ and RLCSD, in which there was a change in diet from 3 to 5 weeks a.d., the average body weights were 92 and 105 g in the former group and 87 and 96 g in the latter group 4 and 5 weeks, respectively. There was a slight tendency that body weight increased in the groups with an addition of CS than in those without an addition of CS.

2. Variations of red blood count, hemoglobin concentration, and packed-cell volume, and myelograms in the groups (Chart 3 and Table 1)

The averages of red blood count, hemoglobin concentration, and packed-cell volume (the three collectively referred to as RHP) in the peripheral venous blood were 7.2 millions per cubic millimeter, 9.6 g per 100 ml of blood, and 40 per cent, respectively, before the start of experimental feeding.

RHP were 7.9 millions per cubic millimeter, 12.4 g were per 100 ml, and 46 per cent, respectively, in group RSCCD+Ca++ 5 weeks a.d. They were 8.0 millions per cubic millimeter, 12.1 g per 100 ml, and 47 per cent in group RSCD and 7.8 millions per cubic millimeter, 12.4 g per 100 ml, and 46 per cent in group RSCD+Ca++ 5 weeks a.d. In groups RSCCD+Ca++, RSCD, and RSCD+Ca++, RHP revealed a tendency to increase gradually until 5 weeks a.d., but there were no significant differences in any value of RHP among these groups. No remarkable changes were found either in the ratio of myeloids (M/E ratio) in the myelogram among these groups, although the total number of erythroids exhibited a slight tendency to increase more in the groups with an addition of CS than in group
Table 1. Myelogram in the Experimental Groups

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<th>RSCD +Ca++</th>
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Chart 3. Variation in Average Values of Red Blood Count, Hemoglobin Concentration, and Packed-Cell Volume in the Six Experimental Groups

Chart 4. Variation in Average Values of White Blood Count and Percentage of Differential Leukocyte Count in the Six Experimental Groups

Remarks: For legend see Chart 1.
RSCD.

In group RLCD, RHP were 5.4 millions per cubic millimeter, 9.2 g per 100 ml, and 36 per cent 5 weeks a.d., indicating the occurrence of a severe anemia. On the other hand, RPH were 6.3 millions per cubic millimeter, 10.9 g per 100 ml, and 41 per cent, respectively, in group RLCSD, and 6.9 millions per cubic millimeter, 11.5 g per 100 ml, and 45 per cent in group RLCSD+Ca++ 5 weeks a.d. In group RLCD, RHP presented a tendency to decrease gradually until 5 weeks a.d., while in groups RLCSD and RLCSD+Ca++, RHP showed a tendency to increase gradually. The increasing tendency of RHP was a little more conspicuous in group RLCSD+Ca++ than in group RLCD. On the other hand, the M/E ratio in the myelogram exhibited a more remarkable tendency to increase in group RLCSD than in group RSCD. The increasing tendency of the M/E ratio was less pronounced in groups RLCSD and RLCSD+Ca++ than in group RLCD.

3. Variations in total and differential leukocyte count (Chart 4)

In all the rats b.d., the average total leukocyte count was 7.8 thousand per cubic millimeter. The average values were 32.5 per cent for neutrophils, 62 per cent for lymphocytes, 3.5 per cent for eosinophils, and 2 per cent for monocytes in the differential leukocyte count of the rats b.d.

In group RSCCD+Ca++, the total leukocyte count was 8.1 and 8.5 thousand per cubic millimeter 3 and 5 weeks a.d., respectively. In groups RSCD and RSCD+Ca++, it was 8.5 and 8.2, and 8.5 and 10.0 thousand 3 and 5 weeks a.d., respectively, showing no significant difference between the groups. In the differential leukocyte count, there was a slight tendency that neutrophils decreased and lymphocytes increased in groups RSCCD+Ca++ and RSCD+Ca++, but no remarkable changes were observed in group RSCD.

On the other hand, the total leukocyte count decreased gradually in group RLCSD until 5 weeks a.d., when it reached a minimum of 2.4 thousand. It was 6.9 and 6.3 thousand in groups RLCSD+Ca++ and RLCD, respectively. In group RLCD, the variations in percentage of the differential leukocyte count were considered to be connected with an increase in neutrophils and a decrease in lymphocytes and eosinophils. In groups RLCSD and RLCSD+Ca++, eosinophils revealed a tendency to increase.

4. Variations in total calcium, ionic calcium, and inorganic phosphorus in the serum (Chart 5)

The average values were 10.4 mg for total calcium, 4.6 mg for ionic calcium, and 6.0 mg for inorganic phosphorus per 100 ml of serum in the rats b.d.

In group RSCCD+Ca++, the total calcium, ionic calcium, and inorganic phosphorus in the serum were 10.5, 4.7, and 6.3 mg per 100 ml of serum 3 weeks a.d., and 10.6, 4.7, and 5.9 mg 5 weeks a.d. Five weeks a.d., the total calcium, ionic calcium, and inorganic phosphorus were 10.4, 4.7, and 6.2 mg in group RSCD+Ca++, and 10.4, 4.6, and 6.5 mg in group RSCD. There were no remarkable changes in the three values in groups RSCCD+Ca++, RSCD+Ca++, and RSCD until 5 weeks a.d.

As for the total calcium, ionic calcium, and inorganic phosphorus in the serum in group RLCD, they manifested a tendency to decrease gradually. The values determined were 9.4 mg for total calcium, 4.3 mg for ionic calcium, and 6.1 mg for inorganic phosphorus on the first day of the experiment.
calcium, 3.6 mg for ionic calcium, and 5.2 mg for inorganic phosphorus 3 weeks a.d., and 7.4 mg for total calcium and 2.9 mg for ionic calcium per 100 ml of serum 5 weeks a.d. The inorganic phosphorus could not be measured 5 weeks a.d. These three values were determined in groups RLCSD and RLCSD+Ca++ 5 weeks a.d. As a result, total calcium was 8.9 mg per 100 ml of serum, ionic calcium 3.9, and inorganic phosphorus 5.2 in group RLCSD, and total calcium 9.0, ionic calcium 3.9, and inorganic phosphorus 5.5 in group RLCSD+Ca++.

5. Variations in total protein and the protein fractions (Chart 6)

The average values were 6.2 g per 100 ml of serum for total protein, 46.8 per cent for the albumin fraction, and 53.2 per cent for the globulin fraction in the percentage of total serum protein, and 0.88 for the albumin/globulin (A/G) ratio in the rats b.d.

The total proteins were 6.7, 6.5, and 6.7 g per 100 ml of serum in groups RSCCD+Ca++, RSCCD+Ca++, and RSCD, respectively, 5 weeks a.d., no remarkable changes being shown among the three groups. No marked difference was present in variations of the protein fractions in percentage between groups RSCCD and RSCCD+Ca++ until 5 weeks a.d. In group RSCD+Ca++, albumin and A/G ratio revealed a tendency to increase 5 weeks a.d.

The average value of the total protein was 5.2, 5.5, and 5.7 g per 100 ml of serum in groups RLCDD, RLCSD, and RLCSD+Ca++ respectively, 5 weeks a.d. In variations of the protein fractions in percentage, albumin and A/G ratio exhibited a remarkable tendency to decrease and alpha-globulin a tendency to increase in group RLCDD until 5 weeks a.d.

Chart 6. Variation in Average Values of Total Protein and Protein Fractions in Serum in the Six Experimental Groups

![Chart 6: Variation in Average Values of Total Protein and Protein Fractions in Serum in the Six Experimental Groups](chart6.png)

Remarks: For legend see Chart 1.
Clinical Studies on Serum Ionic Calcium in Domestic Animals

On the other hand, albumin and A/G ratio showed a tendency to increase and alpha-globulin 5.7 g per 100 ml of serum in groups RLCD, RLCSD, and RLCSD+Ca++, respectively, 5 weeks a.d. In variations of the protein fractions in percentage, albumin and A/G ratio exhibited a remarkable tendency to decrease and alpha-globulin a tendency to increase in group RLCD until 5 weeks a.d. On the other hand, albumin and A/G ratio showed a tendency to increase and alpha-globulin a tendency to decrease in groups RLCSD and RLCSD+Ca++ 5 weeks a.d. Such tendency was more conspicuous in group RLCSD+Ca++ than in group RLCSD.

6. Histopathological findings in the groups five weeks a.d. (Table 2)

a. Findings in group RSCCD+Ca++:
The form, the function, and the tissue structure were normal and regular in hepatic cells and Kupffer's stellate cells, as was the case with groups RSCD and RSCD +Ca++. In the group, the thyroid gland revealed a slight tendency to be in the state of hyperfunction, as compared with that in group RSCD, and had small follicles which were more remarkable than large mature ones in some portions of the organ. The bone marrow was in the state of slight hyperfunction of hematopoiesis with an increase in number of megakaryocytes.

b. Findings in group RLCSD+Ca++:
The structure of liver lobules, the form of hepatic cells, and the stainability of the cytoplasm of these cells were almost normal in general. In some portions of the liver, Kupffer's stellate cells were swollen a little. The thyroid tissue contained almost the same numbers of large and small follicles scattered in it, showing a deep stainability in the small follicles. The bone marrow was almost normal, although there was a tendency of slight hyperfunction in hematopoiesis.

c. Findings in group RLCD: Hepatic cells and Kupffer's stellate cells were found a little swollen in general. The liver tended to stain deeply in the centrolobular region. There was a tendency that the degree of changes in liver tissues was higher in group RLCD than in groups RLCSD and RLCSD+Ca++. In the thyroid gland, large mature follicles were clear for most part, and small ones hardly found. The large follicles contained small quantities of colloid which stained very light. The bone marrow was filled with fat, containing a decreased number of cells. When blood smears from the bone marrow were examined, the myelogram exhibited a tendency that the organ was in a hypofunctional stage of hematopoiesis.

**DISCUSSION**

In the organism, calcium can be found not only in such hard tissues as bone and tooth, but also in the basic structure of cells in the liver, blood vascular tissue and serum throughout the body, in a form of combination with protein. Calcium in the body moves constantly between the blood and bone, and the younger the organism is, the faster it moves. Many investigators have studied the body reaction to a low calcium diet in animals. The authors noticed that the metabolism in an animal fed a severely low calcium diet for a certain period of time was in abnormal conditions, and that hypo-ionic calcemia in the body was closely related to the decline of body defence capacity against an
infection\textsuperscript{22}. Moreover, the authors have drawn an inference that there is a relationship between the decline of SIC and the dysfunction of the liver. This inference is based on findings that hypo-calcemia is induced in the diseases bringing about damage to the liver and in those causing anemia\textsuperscript{16-18,21}, and that a decline in hematopoiesis is observed as a main symptom, like anemia, in growing animals when they have been fed a severely low calcium diet\textsuperscript{19-20}.

In young rats maintained on a severely low calcium diet in this experiment, the body weight increased slightly immediately after the beginning of feeding of this diet, and then showed a tendency to decrease gradually until 5 weeks a.d. As was reported by Fukushima\textsuperscript{83}, it is said that the balance of calcium level in the serum is kept with in a normal limit by the aid of ionic calcium which is released from the bone calcium when calcium intake becomes insufficient in the animal maintained on a low calcium diet. Boelter\textsuperscript{8} stated that the serum calcium and bone calcium in animals maintained on a severely low calcium diet decreased to less than one-half of the value determined in animals fed a regular diet. The authors could not notice the movement of bone calcium exactly, as they did not examine the calcium in this experiment. Nevertheless, they observed a tendency that SIC decreased remarkably and the bone became conspicuously brittle. From these results by the authors, as well as those by the previous investigators\textsuperscript{1-4,8}, the authors have come to draw a main conclusion that the gradual decrease in body weight which appeared after the feeding of a severely low calcium diet was caused by hypoplasia in bone decalcification.

In the rats of group RLCSD which showed a gradual decrease in body weight, there was a tendency that the body weight increased gradually in rats the feeding of which was switched from the first diet to the standard calcium diet containing 414 mg of calcium per 100 g (RLCSD) or to the high calcium diet (RLCSD+Ca\textsuperscript{++}) from 3 to 5 weeks a.d. The increasing tendency of the body weight was more remarkable in group RLCSD+Ca\textsuperscript{++} than in group RLCSD. The hardness of bone and the formation of bone marrow were improved remarkably in the rats of groups RLCSD and RLCSD+Ca\textsuperscript{++}, as compared with those of group RLCSD.

The living body under calcium deficiency has an action to stimulate the calcium absorption from the calcium intake in the diet to the body\textsuperscript{8,7}. It is known that much Ca\textsuperscript{45} is absorbed from the bone in rats maintained on a low calcium diet. The excretion of Ca\textsuperscript{45} into the urine and feces is less in these rats than in those on a regular diet\textsuperscript{8}. When the amount of calcium intake is small, the body has an ability to increase the amount of calcium absorbed from the diet through the intestinal canal\textsuperscript{8,7}, and the increasing calcium in the blood is all ionic calcium\textsuperscript{8}. Calcium moves constantly in the body between the blood and the bone; the younger an organism is, the faster calcium moves in it\textsuperscript{8}. It is well known that the development of rickets depends to some extent upon the ratio of calcium to phosphorus in the diet\textsuperscript{2,7,26}. If the intake of phosphorus is low, absorption is blocked by the formation of calcium phosphate in the intestine. Therefore, rickets can be cured in some cases simply by restoring the normal ratio of one element to the other, or two parts of calcium to one part of phosphorus. When rats maintained on a severely low calcium diet were fed a standard calcium diet or a high calcium diet, there was a tendency that their body weight began to increase gradually, and the conditions of their bone could be improved more conspicuously by the administration of the high calcium diet (that is, the standard calcium diet plus CS) than that of the standard calcium diet. It is said that it is impossible for an animal to absorb over 30 to 50 per cent of the total amount of calcium in a diet, even if the animal is kept under adequate conditions\textsuperscript{27}. In this experiment, CS, which is a suitable form of calcium for being absorbed into the body, was administered with the diet. This type of feeding may have made it easy to fulfill the demand of calcium in the body through the body reaction. In consequence, the authors presumed that the
administration of CS might be necessary for the animal maintained on a severely low calcium diet so that a sufficient quantity of calcium in any form could be obtained from a diet containing an adequate amount of calcium.

Total and ionic calcium, total protein, the albumin fraction, and A/G ratio in the serum decreased gradually in the rats of group RLCD, while they showed a tendency to increase gradually in the rats of groups RLCSD and RLCSD+Ca++, in which the first diet was shifted to a standard calcium diet and a high calcium diet, respectively, from 3 to 5 weeks a.d. They exhibited a more remarkable tendency to increase in the rats fed the high calcium diet (that is, the standard calcium diet with addition of CS) than in those fed the standard calcium diet. The rats fed the severely low calcium diet manifested the dysfunction of the liver, although there was an individual difference in the degree of such dysfunction. It seemed that the damage of the liver might be reduced by the feeding of the standard calcium diet with addition of CS. It has been confirmed that liver damage gives rise to changes in the serum protein fractions and induces a decrease in albumin and total serum calcium, while there is a tendency that albumin and total calcium increase with the progress in recovery of liver damage. When the adsorption of Ca++ was examined in rats with liver damage, more Ca++ was found in the blood, the liver, and the bone than in those organs of normal rats; especially Ca++ in the liver was more than twice as much as that in the liver of a normal rat. The total serum calcium was maintained at a low level over a long period of time when determination was caused in the basic structure of the endothelial cells of blood capillaries in the liver. Administration of calcium had a favorable effect on animals with liver damage. Feeding of a ration containing low calcium induced critical conditions in such animals. The authors have also reported that they obtained the same results from their experiments in a series of studies. In animals which had been fed a severely low calcium diet and which were accordingly under hypocalcemia, total serum protein, albumin, and SIC presented a tendency to take a favorable turn to increase gradually by the administration of a standard calcium diet with addition of CS.

When the rats of group RLCD maintained on a severely low calcium diet were examined for hematopoietic capacity producing red and white blood cells, the following changes were observed: a decrease in RHP and white blood count, an increase in percentage of neutrophils, a decrease of lymphocytes and eosinophils in the differential leukocyte count, and the hypofunction of the bone marrow and thyroid gland. On the other hand, in the rats of group RLCSD+Ca++ which began to be fed a high calcium diet 3 weeks a.d., there was a tendency that RHP and white blood count increased gradually from this time on to return to the normal values, and the bone marrow and thyroid gland were in a slightly hyperfunctional state, as compared with those in group RLCD. As was mentioned in their previous papers quoted above, the authors have pointed out that the body function on hematopoiesis was closely connected with ionic calcium in the body, since an addition of CS might give rise to hyperfunction on hematopoiesis, and that anemia was found in the body of animals suffering from insufficiency of calcium intake for a long time. The factors to adjust hematopoiesis are exceedingly complex and multiphasic. Recently, however, it has been made clear that thyroid hormone, humoral factors, and endocrine regulation are concerned with hematopoiesis. It is known that the principle of hematopoiesis in the bone marrow is the mitosis in the monophasis of the red blood cells and in the diphases of the white blood cells. It is also clear that the adaptation mechanism of hematopoiesis is closely connected with the autonomic nervous system and endocrinum in the body. Wide variation in the hematocyte system should be indicated as a manifestation of whole body reaction in the internal environmental adaptation to external stressor. It means the incapacity of adaptation to the internal environment when the total
leukocyte count decreases by the hypofunction of hematopoiesis in the bone marrow\(^9\). As mentioned in the theories of Schilling\(^{24}\) and Hoff\(^{5}\), the RHP and the total leukocyte count of the rats in group RLCD showed a tendency of increase gradually to return to the normal values when these animals were fed a standard calcium diet with addition of CS, instead of a severely low calcium diet. This tendency might be thought as a reflection and improvement of adaptation capability of the body to external stressors. Taking such range of variation on hematopoiesis in groups RLCD and RLCSD+Ca\(^{++}\) into consideration, it must be concluded that in these groups there is only a suggestion of reduction in the body defence capability. This can be seen in the results of the significant difference in the body reaction of rats infected with *Ancylostoma caninum*, as was already reported by the authors\(^{20}\).

On the other hand, so far as healthy rats in the growing period are concerned, there was a tendency that the body weight increased more distinctly in rats maintained on a high calcium diet than in those maintained on a standard calcium diet alone. As for the variation of various types of metabolism in the animals which had been examined by the authors, there was hardly any significant difference between the two feeding groups. The factors which exert influence on the absorption of calcium in healthy animals are the consistency in the intestinal canal, the pH value, some substances in feedstuff, and vitamin D\(^{25,7,26}\). Even in the case of a diet so prepared as to contain a proper quantity of soluble calcium salts in the finest condition, it seems impossible for the animal to absorb over 50 per cent of the calcium contained in the diet\(^{21}\). It is beyond question that calcium absorption is under the influence of various factors, and, if the other conditions are identical, the coefficient of absorption of calcium should be different from the quantity of calcium demanded in the body. In the case of a young animal which is in the early stage of growth, it is undoubtful that the animal needs to take much calcium for the development of its physical conditions. As calcium metabolism is so multiphasic, even when the animal has been given the same calcium intake per kg of body weight, it should be dissimilar by individual variance. Above the beyond these factors which exert influence upon absorption and excretion of calcium, individual activity, age, and sex should be predominant causes for the demand of calcium in the body. So it is very difficult to determine any standard quantity of calcium exactly required by growing animals. In consequence, it is not easy to decide whether 414 mg of calcium per 100 g of feed, which was given growing rats as a standard calcium diet, was a proper quantity to meet the body demand in the growing period or not. It may be thought, however, that the body reaction to absorb calcium was made very active by the administration of CS. Moreover, the quantity of calcium absorbed from the intestinal canal is quite limited even under most favorable conditions. Therefore, the joint administration of CS, which is a suitable form to be absorbed, may be a very important way to supply calcium in accordance with the body demand. Taking the results obtained from all experiments in the present series of studies and the theories mentioned above into consideration, the authors have come to infer that care must be taken in carrying out the method of calcium administration in order to obtain a sufficient quantity of calcium in the periods of growth, pregnancy, and lactation. It is so difficult to determine any appropriate conditions of calcium absorption which are controled by some multiphasic mechanism.

**SUMMARY**

Three groups of albino rats were fed a standard calcium diet (RSCD), a high calcium diet (RSCCD+Ca\(^{++}\)), and a severely low calcium diet (RLCD), respectively. Three of the 9 rats of group RSCD were fed the high calcium diet for a period of 2 weeks from
3 to 5 weeks after the beginning of experimental feeding (a.d.) (RSCD+Ca++). The rats of group RLCD were divided into three subgroups of 3 rats each, which were fed the standard calcium diet (RLCSD), the high calcium diet (RLCSD+Ca++), and RLCD, respectively, from 3 to 5 weeks a.d.

In group RLCD, the average of body weight, red blood count, hemoglobin concentration, and packed cell volume (the preceding three items collectively referred to as RHP), total leukocyte count, serum ionic calcium (SIC), and total serum protein and albumin decreased gradually until 5 weeks a.d. In groups RLCSD and RLCSD+Ca++, these constituents showed a slight tendency to increase gradually a.d. This increasing tendency was more remarkable in group RLCSD+Ca++ than in group RLCD. In histopathological examination, the liver showed a certain degree of dysfunction and the bone marrow and thyroid gland a slight hypofunction in group RLCD. There was a tendency that these changes were improved slightly in groups RLCD+Ca++ and RLCSD.

In the rats which had been fed the diet of RSCD+Ca++, the body weight showed a tendency to increase more distinctly than in those of group RSCD, as was seen in those of group RSCD+Ca++, but no significant differences were found in body weight among the three groups. Although there were slight differences in body weight among the three groups, no remarkable changes were found in any group in clinical and histopathological examinations.

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家畜血清透析性カルシウムに関する臨床学的研究

X. 低 Ca 食餌飼育ラットに対する Ca** 液経口投与の効果

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発育期の雄名若ラットを、標準 Ca 食餌 (RSCD), 極端な低 Ca 食餌 (RLCD), および高 Ca 食餌 (標準 Ca 食餌 + Ca**, 液, RSCCD+Ca**) の3群に、実験開始と同時に大別して、それぞれの実験食で5週間飼育した。各実験食飼育3週目から、RSCD 群を高 Ca 食餌 (RSCD+Ca**) と RSCD の2群に分け、また RLCD 群を高 Ca 食餌 (RLCD+Ca**)、標準 Ca 食餌 (RLCD) と RLCD の3群にして、5週目までの2週間を、それぞれの実験食餌で飼育した。

RLCD 群ラットでは、体重、赤血球数、血色素量、白血球数、血清透析性 Ca、血清総蛋白量およびアルブミンは、5週目まで逐次的に減少した。RLCD+Ca** と RLCD の2群では、各食餌飼育後、体重、赤・白血球数、血清総蛋白量およびアルブミンが、軽度ながら増加する傾向を認めた。その傾向は、RLCSD+Ca** 群がRLCSD 群よりもやや著明であった。同様に高食食の影響で、RLCSD 群では、肝に軽度の障害および甲状腺廃機能の減退を認めた。RLCSD+Ca** および RSCD 群では、それらは著しく差があるが、全体的に改善されつつあることを知った。
一方、RLCSD+Ca** で発育期のラットを飼育すると、RSCD 飼育ラット群よりも、軽度であるが、体重は増加する傾向を示した。また、RSCD+Ca** 群においても、RLCSD 群に比較して、体重がやや増加する傾向を認めた。これらの3群において、体重増加の度合いに、軽度ながら差はあったが、他の他臨床および組織学的所見では、ほとんど差を認めることができなかった。