CLINICAL STUDIES ON SERUM IONIC CALCIUM IN DOMESTIC ANIMALS

VII. EFFECT OF PERORAL ADMINISTRATION OF CALCIUM ON RABBITS WITH LIVER DAMAGED EXPERIMENTALLY

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To elucidate what influence the variation of serum ionic calcium (SIC) might have over the body reaction of animals injected intravenously with calcium ion solution (CS) as a primary factor or an external force, the authors studied the body reactions of dogs and rabbits, healthy or affected with liver damage, and reported the results in part VI of this series of investigation²³⁾. In that study, SIC showed a passing increase after an intravenous injection of CS, and then returned rapidly to the value before injection. In the body, variation in the erythropoietic system, histological evidence of the hyperfunction of the thyroid gland and bone marrow, and an increase in red blood cells in the peripheral venous blood were observed. From these results, the authors came to a conclusion that the various variations which occurred as the body reaction to the intravenous injection of CS were the expression of a control mechanism in accordance with the adaptation in the body.

The present study was carried out to determine a body adaptation reaction of healthy rabbits and dogs treated by oral administration of CS, and rabbits with the liver damaged experimentally were examined for the body reaction after they had been fed diets containing different amounts of calcium. In consequence, interesting results were obtained, so that they might be used as a step to clarifying the significance of the influence upon the animal body.

MATERIALS AND METHODS

1. Animals used

Twenty-four young male rabbits, weighing about 2 kg each, which had been proved to be in apparently good health by general blood examination, were used for experiments. In order to maintain definite conditions of feeding, they were given 200 g of a standard diet for rabbits (product of the Oriental Co., Tokyo) and 300 ml of tap water per head per day for 2 weeks prior to the experiments.

Five apparently healthy adult dogs, weighing approximately 10 kg each, were fed a regular ration and tap water freely for 2 weeks before they were used for the experiment.

2. Measurement methods

All the measurements were performed precisely in accordance with the established procedure of the methods set forth in the previous reports¹⁸⁻²³⁾.

3. Experimental methods

a. Oral administration of calcium ion solution in healthy rabbits and dogs: In order to study the body reaction to the oral administration of CS, some healthy rabbits were given

a single dose of 10 ml of CS containing 75 mg of calcium ion per 100 ml per kg of body weight; others were administered perorally with 10 ml of the same solution per kg of body weight daily for 7 days. Some healthy dogs were administered perorally with a single dose of 10 ml of CS, and others with the same dose of the same solution by the same route daily for 3 days.

b. Oral administrations of calcium ion solution in rabbits with the liver experimentally damaged (Chart 1): To study the body reaction to the oral administration of CS in rabbits

Chart 1. Scheme of the Second Experiment

Group*	Before	1	2	4
CCl ₄ INJECTED		0.01 ml each		(weeks)
SCD				
HCD				
LCD				
NDD				
HEALTHY CONT				

* SCD, standard calcium diet; HCD, high calcium diet; LCD, low calcium diet; NDD, nutritional deficiency diet; and CONT, healthy control.

the liver of which had been damaged experimentally, rabbits in such state were injected subcutaneously with three doses of a net quantity of 0.01 ml each of carbon tetrachloride per kg of body weight. The inoculum was a 0.2 per cent solution of carbon tetrachloride in olive oil. The rabbits were divided into 4 groups after the first injection with carbon tetrachloride and examined for 4 weeks. The 4 groups were fed a standard calcium diet (SCD; containing 1.7 % of Ca), the standard calcium diet+CS (HCD; 1.7% of Ca+225 mg of Ca⁺⁺), a low calcium diet (LCD; 0.7% of Ca), and a nutritional deficiency diet

(NDD; 0.5% of Ca), respectively. As the control to these experimental groups, a group of two healthy rabbits (CONT) was fed SCD for the whole period of an experiment. The rabbits of all groups were given 300 ml of tap water per head per day, except those of group HCD. Those of group HCD was given 300 ml of CS, instead of the tap water.

RESULTS

A. Oral administrations of calcium ion solution in healthy rabbits and dogs

1. Oral administration of a single dose of 10 ml/kg of calcium solution

With a view to detect a body reaction to the oral administration of CS, observation was carried out on the SIC of rabbits during 24 hours. When a male rabbit, weighing 2.1 kg, was administered with 10 ml of CS *per os*, it contained 75 mg of Ca⁺⁺ per 100 ml of solution, per kg of body weight (Table 1), and showed a gradual decrease in SIC until 6 hours after the first administration (a.a.). After that, SIC increased gradually in it, showing a tendency to recover 24 hours a.a.

The red blood count, hemoglobin concentration, and packed-cell volume (these three entities being referred to as RHP) decreased gradually until 6 hours a.a., and then increased gradually to return to the normal values 24 hours a.a. No conspicuous variation was observed in the leukocyte count a.a. In the differential leukocyte count, heterophils decreased slightly and lymphocytes increased in percentage.

2. Peroral administration of 10 ml/kg of calcium solution daily for 7 days

When a male rabbit, weighing 2.0 kg, was administered *per os* with 10 ml of CS per kg of body weight daily for 7 days (Table 2), it revealed a slight decrease in SIC 24 hours a.a. Then SIC showed a tendency to increase gradually by the 14th day a.a. and returned to the normal value after that. The RHP increased gradually by the 14th day a.a. The leukocyte count changed a little. There were no definite changes in the differential leukocyte count.

In order to elucidate the influence of calcium on the internal organs, specimens were collected from the tissues of liver, kidney, spleen, pancreas, bone marrow, and thyroid of an animal administered with CS daily for 7 days. In the liver, hepatic cells and Kupffer's stellate cells were in normal state, and the function and the tissue structures were also normal (Fig. 1). In the kidney, glomeruli and uriniferous tubules were normal in tissue

Table 1. Influence of an Oral Administration of Ca^{++} on Serum Ionic Calcium in a Healthy Rabbit

Time in hours		Before admin.	After administration				
			1/2	2	6	12	24
SERUM							
Calcium	mg/dl						
Total		12.1	11.8	11.8	11.7	12.0	12.3
Ionic		4.8	4.8	4.6	4.4	4.8	4.7
McLean		6.0	5.9	6.0	5.9	6.2	6.3
Ion/T. Ca	%	39.7	40.7	39.0	37.6	40.0	38.9
Protein	g/dl						
Total		5.8	5.8	5.6	5.6	5.4	5.6
BLOOD							
R.B.C.*	10 ⁶	5.8	5.4	5.0	5.0	5.6	6.0
Hb.	g/dl	11.2	11.1	10.8	11.0	11.3	11.5
Ht.	%	41	40	38	39	40	40
W.B.C.**	10 ³	7.9	7.1	9.2	8.6	7.9	6.4
Hetero.	%	49			42		40
Eosin	%	2			2		1
Lymph.	%	46			55		57
Mono.	%	2			1		2
Baso.	%	1			0		0

Remarks: The dose of calcium ion solution administered was 10 ml per kg of body weight and contained 75 mg of Ca^{++} per 100 ml.

* R.B.C., red blood count; Hb., hemoglobin; and Ht., hematocrit.

** White blood count; heterophils, eosinophils, lymphocytes, monocytes, and basophils in the order listed.

Table 2. Influence of Oral Administrations of Ca^{++} on Serum Ionic Calcium in a Healthy Rabbit

Time in days		Before admin.	After administration				
			1	3	7	14	21
SERUM							
Calcim	mg/dl						
Total		11.4	11.3	11.4	11.5	12.0	11.6
Ionic		4.2	4.1	4.4	4.6	5.0	4.6
McLean		5.9	5.9	5.8	6.0	6.1	5.8
Ion/T. Ca	%	36.9	36.2	38.6	40.0	41.6	39.6
Protein	g/dl						
Total		5.4	5.2	5.6	5.4	5.6	5.6
BLOOD							
R.B.C.*	10 ⁶	4.8	5.0	5.4	6.5	6.5	6.9
Hb.	g/dl	8.4	8.4	9.8	11.0	11.5	11.9
Ht.	%	38	38	39	41	43	44
W.B.C.*	10 ³	10.8	7.2	8.4	6.7	5.3	8.5
Hetero.	%	30	27	21	33	34	38
Eosin.	%	1	2	2	1	1	2
Lymph.	%	65	68	74	63	60	57
Mono.	%	2	3	2	3	4	1
Baso.	%	2	0	1	0	1	2

Remarks: The rabbit was administered with 10 ml of solution per kg of body weight by the oral route daily for a week.

* See Table 1.

Table 3. Influence of an Oral Administration of Ca^{++} on Serum Ionic Calcium in a Healthy Adult Dog

Time in hours		Before admin.	After administration				
			1/2	2	6	24	48
SERUM							
Calcium	mg/dl						
Total		11.5	10.9	10.5	11.0	11.6	11.7
Ionic		4.5	3.5	3.1	3.9	3.4	4.0
McLean		5.2	4.9	4.8	5.0	5.4	5.6
Ion/T. Ca	%	39.1	32.1	29.5	35.4	31.1	34.1
Protein	g/dl						
Total		6.8	6.6	6.7	6.7	6.5	6.6
BLOOD							
R.B.C.*	10 ⁶	6.0	5.4	5.0	5.7	6.0	5.9
Hb.	g/dl	13.1	11.9	11.9	12.0	12.8	12.8
Ht.	%	42	39	38	40	42	41
W.B.C.*	10 ³	9.0	9.2	9.8	9.5	9.5	10.1
Neutro.	%	53				42	51.5
Eosin.	%	21.5				30.5	31.5
Lymph.	%	19.0				22.5	14.5
Mono.	%	6.0				5.0	2.5
Baso.	%	0				0	0
Mean nucleus of neutrophils		2.20				2.09	2.17

Remarks: The dose of calcium ion administered was 10 ml of solution per kg of body weight. The dog weighed 10.2 kg.

* See Table 1.

Table 4. Influence of Oral Administrations of Ca^{++} on Serum Ionic Calcium in a Healthy Adult Dog

Time in hours	Before admin.	After the first administration													
		1/2	2	6	24	24½	26	30	48	48½	50	54	72	96	
SERUM															
Calcium	mg/dl														
Total		12.3	12.0	11.2	11.1	11.6	11.1	11.3	11.4	11.6	11.3	10.5	11.0	11.3	11.6
Ionic		4.9	4.8	4.7	4.3	4.6	4.3	4.2	4.5	5.0	4.9	4.0	4.9	4.4	4.9
McLean		5.8	5.8	5.4	5.2	5.4	5.2	5.4	5.4	5.5	5.3	5.0	5.2	5.4	5.5
Ion/T. Ca	%	39.8	40.0	41.9	38.7	39.6	38.7	37.1	39.4	43.1	43.3	38.1	44.5	38.5	42.2
Protein	g/dl														
Total		6.4	6.2	6.2	6.4	6.6	6.4	6.2	6.2	6.4	6.4	6.2	6.4	6.2	6.4
BLOOD															
R.B.C.*	10 ⁶	6.4	6.3	6.2	6.2	6.7	5.8	6.1	6.2	6.3	5.8	5.6	5.9	6.1	6.4
Hb.	g/dl	13.3	12.6	13.0	13.1	13.3	13.1	12.3	13.2	13.8	12.8	12.2	13.1	13.1	13.3
Ht.	%	45	42	41	45	45	41	41	43	42	40	39	41	43	42
W.B.C.*	10 ⁸	11.3	11.2	10.5	11.5	10.4	8.5	10.2	10.2	8.9	10.2	9.7	9.0	10.1	10.5
Neutro.	%	58.5				57.5				53.5				42.0	51.5
Eosin.	%	24.5				24.0				21.5				30.5	20.5
Lymph.	%	13.0				14.5				19.0				22.5	14.5
Mono.	%	4.0				3.5				6.0				5.0	2.5
Baso.	%	0				0.5				0				0	0
Mean nucleus of neutrophils		2.20				2.06				2.05				2.09	2.13

Remarks: The dog was administered with 10 ml of solution per kg of body weight by the oral route daily for 3 days.

* See Table 1.

Table 5. Influence of Oral Administrations of Ca^{++} on Serum Ionic Calcium in a Healthy Adult Dog

Time in hours	Before admin.	After the first administration													
		1/2	2	6	24	24½	26	30	48	48½	50	54	72	96	
SERUM															
Calcium	mg/dl														
Total		11.9	11.4	11.1	10.7	11.0	10.7	10.0	11.2	11.4	10.3	10.4	11.0	11.8	11.8
Ionic		4.6	4.3	4.0	4.1	4.4	4.0	4.0	4.4	4.6	4.0	4.2	4.8	4.7	4.8
McLean		5.4	5.3	5.2	5.0	5.1	5.0	4.7	5.2	5.3	4.8	4.8	5.1	5.5	5.5
Ion/T.Ca	%	38.6	37.7	36.1	38.3	40.0	37.3	40.0	39.2	40.3	38.8	40.3	43.6	39.8	40.6
Protein	g/dl														
Total		6.8	6.6	6.4	6.4	6.6	6.4	6.4	6.6	6.6	6.4	6.6	6.4	6.6	6.6
BLOOD															
R.B.C.*	10 ⁶	6.9	6.1	5.7	6.9	6.6	5.8	5.6	6.2	6.0	5.7	5.2	5.9	6.0	6.8
Hb.	g/dl	13.3	13.0	12.8	13.4	13.6	13.0	12.8	13.3	12.8	12.8	12.3	12.5	13.0	13.6
Ht.	%	44	43	40	44	45	43	40	44	42	40	39	41	43	43
W.B.C.	10 ³	9.7	8.0	8.0	9.3	10.1	9.1	8.7	10.5	9.0	8.2	9.7	10.0	9.4	11.0
Neutro.	%	46.5				49.0				45.0				32.0	56.0
Eosin.	%	13.5				20.0				25.0				30.0	20.0
Lymph.	%	30.5				23.5				24.5				35.5	20.5
Mono.	%	9.5				7.5				5.5				2.5	3.5
Baso.	%	0				0				0				0	0
Mean nucleus of neutrophils		2.01				2.00				2.04				2.02	2.04

Remarks: The dog was administered with 10 ml of solution per kg of body weight by the oral route daily for 3 days.

* See Table 1.

structure. In the thyroid tissue, immature follicles were a little larger in number than those in the normal thyroid tissue (Fig. 2), and there was a slight evidence of hyperfunction. A hematopoietic hyperfunction was observed slightly in the red bone marrow (Fig. 3).

3. Oral administration of a single dose of 10 ml/kg of calcium solution

A female adult dog, weighing 10 kg, was administered *per os* with 10 ml of CS, which contained 75 mg of Ca^{++} per 100 ml of solution, per kg of body weight (Table 3). In it, SIC decreased gradually until 2 hours a.a. and then increased slightly, showing a tendency to recover.

The RHP showed a tendency to decrease slightly until 2 hours a.a. and then increased gradually to return to their values determined before the administration. The leukocyte count varied little. In the differential leukocyte count, there was a tendency that neutrophils decreased, and lymphocytes and eosinophils increased a little in percentage 24 hours a.a.

4. Peroral administration of 10 ml/kg of calcium solution daily for 3 days

Three male adult dogs, weighing approximately 10 kg, were administered *per os* with 10 ml of CS containing 75 mg of Ca^{++} per 100 ml of solution, per kg of body weight daily for 3 days (Tables 4 and 5). SIC and serum total calcium decreased gradually from 2 to 6 hours a.a. After that, they increased gradually, showing a tendency to return to their values determined before the administration. After the second and the third administration, they went down again until 2 hours a.a., showing such a gradual increase to recover as seen after the first administration.

The RHP decreased gradually a.a., reaching their minima after 2 hours. After that, they began to increase gradually, showing a tendency to recover. After the second and the third administration, they showed the same tendency as revealed after the first, the variation being less remarkable than that after the first administration. No distinct changes were

Chart 2. Changes in Red Blood Count, Hemoglobin Concentration, and Packed-Cell Volume in All Groups

Remarks: CONT, healthy control; HCD, high calcium diet; SCD, standard calcium diet; LCD, low calcium diet; and NDD, nutritional deficiency diet. All the groups were given three doses of 0.01 ml each per kg of body weight every other day, except the control group.

B* before administration.

Chart 3. Changes in the Mean of White Blood Count and That of Differential Leukocyte Count

Remarks: C, CONT; S, SCD; H, HCD; L, LCD; and N, NDD. See also Chart 2.

noticed in the total leukocyte count. In the differential leukocyte count, there was a tendency that neutrophils decreased and lymphocytes increased both gradually in percentage.

B. Oral administration of calcium solution in rabbits with the liver experimentally damaged with carbon tetrachloride

1. Variations in red blood count, hemoglobin concentration and packed-cell volume (Chart 2)

a. Group HCD: In a week after the first injection of carbon tetrachloride, the RHP showed a tendency to increase slightly, as compared with their values determined before the injection (b.i.). They exhibited a tendency to increase 2 and 4 weeks after the first injection (a.i.).

b. Group SCD: The red blood count decreased gradually until 2 weeks a.i. After that, it increased slightly, showing a tendency to return to its value determined b.i. The hemoglobin concentration showed a mini-

mum value a week a.i., and then increased gradually, presenting a tendency to recover. The packed-cell volume, however, revealed no remarkable increase nor decrease.

c. Group LCD: The red blood count showed a tendency to decrease gradually until 2 weeks a.i., remaining still at a low level 4 weeks a.i. Both hemoglobin concentration and packed-cell volume manifested a tendency to decrease gradually until 4 weeks a.i. and never went up by the end of the experiment.

d. Group NDD: In this group of three rabbits, only one animal was alive 4 weeks a.i. The other two died 6 and 23 days a.i., respectively. The RHP decreased gradually until 4 weeks a.i. The degree of such decrease was the highest of all the groups.

e. Group CONT: In the group of healthy rabbits fed the standard diet, the RHP varied little during 4 weeks.

2. Variations in the white blood count and the differential leukocyte count in percentage (Chart 3)

a. Group HCD: Though the total leukocyte count showed a slight tendency to decrease gradually a.i., the variation was hardly remarkable in general. In the differential leukocyte count, heterophils decreased slightly and lymphocytes increased in percentage.

b. Group SCD: No distinct changes were noticed in the total leukocyte count. There was a slight tendency that lymphocytes increased and heterophils decreased in percentage.

c. Group LCD: There were no definite changes in the total leukocyte count. In the differential leukocyte count, there was a tendency that heterophils increased and lymphocytes and eosinophils decreased a little in percentage.

d. Group NDD: The total leukocyte count showed a tendency to increase slightly. There was a tendency that heterophils increased and lymphocytes and eosinophils decreased gradually in percentage.

e. Group CONT: No conspicuous variations were observed in the total and differential leukocyte count.

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

a. Group HCD: SIC showed a tendency to decrease slightly a week a.i., when the administration of CS was still going on. After that, it increased gradually until 4 weeks a.i. to return to its value determined b.i. The inorganic phosphorus in the serum showed a slight tendency to increase gradually until 4 weeks a.i. In the variations of SIC and inorganic phosphorus, it seemed that there might be inverse relations between the decrease of SIC and the increase of inorganic phosphorus.

b. Group SCD: SIC decreased remarkably a week a.i. and then increased gradually until 4 weeks a.i., showing a tendency to recover. The inorganic phosphorus increased remarkably a week a.i. and then showed a tendency to decrease gradually until 4 weeks a.i. It seemed that there might be inverse relations between the variation of SIC and that of inorganic phosphorus.

c. Group LCD: SIC showed a gradual decrease until 2 weeks a.i. After that, it remained at this low level up to 4 weeks a.i., showing no tendency to increase. The inorganic phosphorus decreased slightly a week a.i. and then increased remarkably 2 weeks a.i. After that, it showed a tendency to decrease slightly until 4 weeks a.i.

d. Group NDD: SIC decreased gradually until 4 weeks a.i., reaching a minimum value 4 weeks a.i., and never increased until death. The inorganic phosphorus showed a tendency to decrease a week a.i., and then increased remarkably until 3 weeks a.i.

e. Group CONT: Total calcium, ionic calcium, and inorganic phosphorus in the serum revealed no remarkable increase nor decrease.

4. Variation in total protein and the protein fractions in the serum (Chart 5)

a. Group HCD: The total serum protein began to increase slightly a week a.i., decreasing slightly 2 weeks a.i. After that, it showed a tendency to increase again until 4 weeks a.i. Of the protein fractions, the albumin fraction decreased in percentage a week a.i., showing

Chart 4. Values of Total Calcium, Ionic Calcium, and Inorganic Phosphorus in Serum in All Groups

Remarks: See Chart 2.

Chart 5. Values of Total Protein and Per Cent Concentrations of Protein Fractions in Serum in All Groups

Remarks: See Chart 2.

a tendency to increase gradually until 4 weeks thereafter. Alpha- and gamma-globulin showed a slight increase a week a.i. and then decreased gradually until 4 weeks a.i. In consequence, the variation of A/G ratio was parallel to that of the albumin fraction in general.

b. Group SCD: The total serum protein showed a slight tendency to increase gradually

Chart 6. Changes in Total Cholesterol in Serum

Remarks: See Chart 3.

Chart 7. Changes in Total Iron in Serum

Remarks: A, supplemented with calcium ion and B, not supplemented with calcium ion. For the other abbreviations see Charts 2 and 3.

until 4 weeks a.i. Of the protein fractions, albumin decreased and alpha- and gamma-globulin increased in percentage a week a.i. After that, there was a tendency that albumin and gamma-globulin increased and alpha-globulin decreased gradually until 4 weeks a.i. The variation of A/G ratio was parallel with that of the albumin fraction in general.

c. Group LCD: The total serum protein decreased a week a.i. and then showed a tendency to increase slightly until 4 weeks a.i. In the protein fractions, there was a slight tendency that the albumin fraction decreased and beta- and gamma-globulin increased a week a.i. Two weeks later, albumin increased gradually and the globulins decreased in percentage.

d. Group NDD: The total serum protein increased slightly until 2 weeks a.i. and then decreased gradually until 4 weeks a.i. The albumin fraction showed a slight tendency to decrease until 2 weeks a.i. and then increased gradually, while the globulins increased gradually until 2 weeks a.i., showing a tendency to decrease 4 weeks a.i.

e. Group CONT: In healthy rabbits, the total serum protein showed a slight tendency to increase gradually until 4 weeks a.i. No change was remarkable in the protein fractions, except in the albumin fraction of a rabbit where there

was a slight tendency to increase gradually.

5. Variations in total cholesterol in the serum (Chart 6)

a. Group HCD: The total cholesterol increased remarkably to three times as large as the normal value a week a.i. After that, it decreased gradually until 4 weeks a.i., and returned to the normal value. The tendency to decrease was more remarkable in this group than in any of the other groups.

b. Group SCD: The total cholesterol increased remarkably a week a.i. and then showed a tendency to decrease gradually until 4 weeks a.i.

c. Group LCD: The total cholesterol increased a week a.i., showing a tendency to decrease gradually until 4 weeks a.i. The decreasing tendency was the least remarkable of all the groups.

d. Group NDD: The total cholesterol showed a remarkable increase until 2 weeks a.i. and decreased slightly 4 weeks a.i. Its value determined 2 weeks a.i. was approximately more than twice as large as those given in the other groups at the same time.

e. Group CONT: In healthy rabbits fed SCD, the total cholesterol revealed no remarkable increase nor decrease.

6. Variations in total iron in the serum (Chart 7)

a. Group HCD: The serum iron increased a week a.i., returning to its value determined before injection 2 weeks a.i. After that, it was again at a high level 4 weeks a.i.

b. Group SCD: The serum iron showed a slight tendency to increase until 2 weeks a.i. and decrease gradually thereafter.

Table 6. Changes in Myelogram

Group*	HEALTHY CONT	CCl ₄ injected			
		HCD	SCD	LCD	NDD
ERYTHROPOIESIS					
Proerythroblasts	1.5	2.7	1.7	1.0	1.0
Macroblasts	0	2.0	0	0	0
Normoblasts	23.5	58.0	39.9	34.3	20.0
Erythroid: Total	25.0	62.6	41.3	35.3	21.0
GRANULOPOIESIS					
Myeloblasts	0.5	0.7	1.3	1.3	5.0
Promyelocytes	2.5	3.7	4.0	4.0	12.0
Myelocytes					
heterophile	4.0	5.6	6.3	6.7	3.0
eosinophile	1.5	2.3	1.3	5.6	1.0
basophile	0	2.7	0.7	0.3	0
Metamyelocytes					
heterophile	8.5	14.0	13.3	11.7	9.0
eosinophile	2.5	2.3	2.7	3.0	2.0
basophile	1.0	2.7	0.7	1.6	1.0
Stab leukocytes					
heterophile	19.5	32.3	30.7	26.0	20.0
eosinophile	0	0	0	0	0
basophile	0	0	0	0	0
Segment leukocytes					
heterophile	16.5	21.7	16.7	19.6	5.0
eosinophile	0	0	0	0	0
basophile	0	0	0	0	0
NON-GRANULOPOIESIS					
Lymphocytes	17.0	11.6	18.0	18.0	15.0
Monocytes	2.0	3.0	3.7	4.0	2.0
Plasma cells	0	0	0	0	0
Myeloid: Total	76.3	100.1	107.4	105.9	79.0
Myeloid/erythroid					
Ratio	3.1	1.6	2.6	3.0	3.8
Megakaryocytes	0.3	1.5	0.9	0	0
Reticulum cells	0	0	0	0	0
Ferrata cells	0	0	0	0	0

* For the abbreviations see the text or Table 7.

by the Oriental Co. for 4 weeks under the same conditions as those of the experimental groups before they were subjected to autopsy.

a. Group HCD: In the rabbits which had received subcutaneous injection of three doses of a net quantity of 0.01 ml each of carbon tetrachloride per kg of body weight, no remarkable change was observed in the liver, as compared with that of a healthy one. When microscopically observed, the structure of the liver lobule, the forms of hepatic cells and Kupffer's stellate cells, and the stainability of the cytoplasm were regular (Figs. 4 and 5). There was no remarkable change in the blood distribution of the liver, as compared with the normal liver. The kidney tissue exhibited a slight ectasia in the centrolobular region, but it presented generally a normal appearance. In the thyroid tissue (Fig. 6), products of small immature follicles were remarkable than those of large mature ones. The colloid in the follicles manifested a very distinct stainability. The tissue of the bone marrow showed

c. Group LCD: The serum iron was at a high level a week a.i. and then decreased gradually until 4 weeks a.i. Its value determined 4 weeks a.i. was approximately half that determined before the injection.

d. Group NDD: The serum iron showed a slight tendency to increase a week a.i. and then decreased remarkably until 4 weeks a.i.

e. Group CONT: In healthy rabbits, the serum iron revealed no remarkable change until 4 weeks a.i. It showed $\pm 5 \mu\text{g}$ per 100 ml of serum as variation limits.

With a view to detect the variation of serum iron under the influence of the Ca^{++} supplement or Ca^{++} non-supplement in the same diets, each group of rabbits which were fed SCD, LCD, or NDD was held under observation for 4.5 weeks to examine changes in serum iron.

When the same diet was used, the serum iron showed a significantly higher level in rabbits fed the Ca^{++} supplement than in those not fed the Ca^{++} supplement in each group.

7. Histological examination in groups HCD, SCD, LCD, and NDD (Tables 6 and 7)

Specimens were collected from such organs as liver, kidney, spleen, pancreas, thyroid gland, and bone marrow of healthy rabbits. They were normal in appearance. The rabbits of the healthy control group (CONT) had been fed the standard diet produced

Table 7. Histopathological Examination on Rabbits with Liver Damage Fed a Low or a High Calcium Diet Daily for Four Weeks

Group*	Organs examined					
	Liver**	Kidney	Spleen	Pancreas	Thyroid gland**	Bone marrow**
Liver damage groups						
HCD	— ~ ±	—	—	—	± ~ +	± ~ +
SCD	± ~ +	—	—	—	— ~ ±	—
LCD	+	—	—	—	— ~ ±	—
NDD	+ ~ ++	—	—	—	—	—
Healthy control group						
CONT	—	—	—	—	—	—

Remarks: Each rabbit was injected with three doses of 0.01 ml each per kg of body weight every other day.

* HCD, high calcium diet; SCD, standard calcium diet; LCD, low calcium diet; NDD, nutritional deficiency diet; and CONT, healthy control fed the standard calcium diet.

** Changes of the liver are indicated by the degree of damage and those of the thyroid gland and bone marrow by functional acceleration as compared with those in the healthy control group.

a tendency to be in hyperfunction with a slight increase of cells (Fig. 7). In the smear preparation of bone marrow, the ratio of myeloids to erythroids was 1.6, while it was 3.1 in the normal rabbit. Therefore, the authors presumed that those histological changes found in the thyroid gland and the bone marrow might be closely related to the hyperfunction of the erythropoietic system. No changes were found in the other organs.

b. Group SCD: The degree of damage of the liver tissue was lower in this group than in group LCD or NDD. A slight infiltration of inflammatory cells was found around Glisson's sheaths (Fig. 8). The stainability was regular in general, although there was a slight tendency that cells in the centrolobular region stained deeply. The kidney tissue did not differ from that of the healthy animal in histological appearance. Large and small follicles, approximately equal in number, were scattered over the thyroid tissue (Fig. 9). The large immature follicles, some of which were filled with colloid, remained normal in stainability and in the epithelial condition. The histological findings of the bone marrow were little different from those of the normal one (Fig. 10), though the erythropoietic system seemed to be in a slight hyperfunctional state, judging from the myeloid/erythroid ratio in the smear preparation.

c. Group LCD: The liver tissue showed an irregular stainability of the cytoplasm, diversity of the nuclear size, and dilatation of the central and pericentral sinusoids (Fig. 11). Sometimes slight swelling was observed in hepatic cells and Kupffer's stellate cells. Slight swelling of glomeruli and uriniferous tubules was observed in a part of the kidney tissue. No other remarkable changes were present in the tissues of the kidney. In the thyroid tissue, large and small follicles, approximately equal in number, were scattered around and the colloid in the follicles was very light in staining and small in quantity (Fig. 12). Histologically, the bone marrow showed a slight cellular hyperplasia, though the variation in blood smear was not different from that of the normal tissue (Fig. 13).

d. Group NDD: In the liver tissue, dilatation was conspicuous in the central and pericentral sinusoids. The hepatic cells and Kupffer's stellate cells exhibited a slight swelling with the irregular stainability of the cytoplasm and pyknotic or vesicular degeneration of the nucleus. Fatty degeneration of hepatic cells was found diffusely in the acini (Fig. 14). A slight swelling was found in glomeruli of the kidney. The thyroid tissue contained mostly large mature follicles and few small ones (Fig. 15). In the bone marrow, the yellow marrow had much fat accompanied by a very few cells (Fig. 16). In the blood smear, a state of

hypofunction in erythropoiesis was observed with an increase in the myeloid to erythroid ratio.

DISCUSSION

In animals intravenously injected with CS, as the authors already reported²⁸⁾, the increase of serum calcium in the circulating blood is caused by that of the ionic calcium. A high level of serum calcium is no more maintained, except for a short period of time, after that SIC shows a tendency to decrease rapidly to return to its normal value. From these findings on serum calcium, the authors presumed that the variation might be an expression of adaptation of the whole body to the injection of CS as an external stressor.

In an animal perorally administered with CS, the variation of SIC showed a slight tendency to decrease gradually from 2 to 6 hours approximately after the administration, though it was different from that of the volume of CS. After that, it increased gradually toward its normal value. In the same experiment, the variation of RHP showed a similar tendency to that of SIC. In an animal administered perorally with CS the reaction of which was over pH 12, this phenomenon was caused by a transient increase in volume of the circulating blood⁸⁾. In consequence, the low levels of SIC measured experimentally do not mean a decrease in total amount of calcium in the whole blood of the body. In general, in an animal administered perorally with CS, calcium is absorbed through the small intestine in a form of calcium ion⁹⁾ and, passing through the portal vein, enters the liver and the systemic blood circulation^{5,29)}. It has been proved by an experiment that calcium ion, after entering the liver through the portal vein, adheres remarkably to the liver tissues²⁵⁾. In healthy rabbits injected with CS daily for 7 days, such damage as presumed to be induced by this adhesion of CS was not found in the histological picture of the liver²⁸⁾. A slight tendency to induce a hyperfunction was observed in the histological pictures of the thyroid tissue and bone marrow. There was also an increase in RHP and lymphocyte count. Besides, no remarkably severe clinical symptoms were found in an animal intravenously injected with CS. In consequence, it has been proved that in healthy animals calcium absorption is controlled to progress or decline by a necessary volume of calcium in the body, as reported by an investigator⁵⁾. Then the authors presumed that a small volume of calcium would be absorbed into the blood even if a large quantity of CS was given perorally to a healthy adult animal.

It is well known¹⁰⁾ that the thyroid function is related to erythropoiesis with regard to the control on body fluids. Peroral administration of CS gave rise to a slight reaction in a series of erythropoietic systems in the body. Therefore, the authors assumed that the variations might be a kind of appearance of the body reaction to CS.

Rabbits with liver damaged experimentally, were fed such diets as HCD (SCD+CS), SCD, LCD, and NDD. The authors could observe that the variations of RHP, bone-marrow picture, serum iron, and histopathological changes of the liver and thyroid tissues in group HCD differed from those in group LCD. MINOT et al.¹⁶⁾ stated that administration of high calcium had a favorable effect on rabbits with the liver damaged by carbon tetrachloride, and that the feeding of a low calcium diet induced critical conditions in such rabbits. MARTIN¹⁴⁾ examined the influence of a low calcium diet on a healthy puppy. He could observe no remarkable hypocalcemia, but recognized distinct changes in various kinds of metabolism of protein, lipids, and glucose in the body. BOELTER³⁾ reported that in animals fed a low calcium diet, serum total calcium and bone calcium decreased remarkably in value to half the values determined in animals fed a standard calcium diet, and that in the former animals, inorganic phosphorus also decreased remarkably after the feeding of the diet and then went up to recover. In the rabbits fed LCD and examined by the authors, SIC in the body

decreased gradually for 2 weeks after injection of carbon tetrachloride, remaining still at a low level 4 weeks a.i., while inorganic phosphorus showed a tendency to increase gradually until 4 weeks a.i. On the other hand, in the rabbits fed HCD in the present experiments, SIC in the body showed a slight decrease for a week a.i. and then increased gradually to return to its value before the injection. In these experiments, total cholesterol in the serum increased remarkably in groups HCD and LCD for a week a.i. After that, in both groups, it decreased gradually until 4 weeks a.i. to return to its normal value, a tendency to decrease being less distinct in group LCD than in group HCD. It is well known that the total cholesterol has a tendency to increase in the case of liver damage. Some authors^{6,14)} reported that in healthy rats fed a low calcium diet, total cholesterol in the serum showed a significant decrease, or no remarkable change, as compared with those fed the standard calcium diet. On the other hand, the authors presume that the difference in variation of serum total cholesterol between animals fed a high calcium diet and those fed a low calcium diet might be related to the severity of liver damage. It was reported that rabbits injected with carbon tetrachloride showed a remarkable decrease in liver calcium, and that the decrease of calcium was correlated to the liver damage²⁵⁾. In rats with the liver damaged by carbon tetrachloride, more than twice as much Ca^{45} the liver absorbed as that in healthy ones, and the absorbed Ca^{45} would be used for the basic structure of the endothelial cells of blood capillaries in the damaged liver¹⁵⁾. In the histopathological findings of the liver obtained this time by the authors, a more deteriorating tendency was found in group LCD than in group HCD. In group NDD, SIC decreased remarkably after the first injection of carbon tetrachloride, reaching its minimum value shortly before death in two of the three rabbits injected during the period of experiment. Histopathologically, the liver of the remaining one rabbits, which survived until 4 weeks a.i., showed the most deteriorated picture of the rabbits of all groups in this experiment.

In protein metabolism in the serum of the rabbits, there were no remarkable differences in total protein and protein fractions between groups HCD and LCD. As changes in the protein fractions of the serum, a transient decrease and increase in the albumin fraction and the corresponding transient increase and decrease in the globulins were observed, this tendency being common in all experimental groups. It is well known that the albumin fraction of the serum shows a tendency to decrease in accordance with the severity of damage of the liver, and that the increase is accompanied by the recovery of liver function. According to FUKUSHIMA⁶⁾, no significant change was found in protein metabolism between animals fed a slightly low calcium diet and those fed a standard calcium diet. There were no remarkable differences in the variation of total protein and protein fractions between groups HCD and LCD. From these results, the authors presumed that similar changes in protein metabolism might be caused in both groups by extremely slight liver damage which had been brought about by injection of carbon tetrachloride and, moreover, that the amount of calcium contained in the low calcium diet might not be large enough to give any influence on protein metabolism in the body.

Significant differences were found in hematopoiesis in the tissues of bone marrow and thyroid gland, RHP, and serum iron between groups LCD and HCD. In group LCD, a tendency was observed that RHP decreased gradually to show a remarkable anemia. On the other hand, in group HCD, RHP and serum iron increased gradually, while an erythropoietic hyperfunction was observed in the myelogram, or the histological picture of the bone marrow, showing an acceleration of thyroid tissue. The presence of nitrogen retention gives the bone marrow an ability to regenerate hemoglobin, and deficiency of thyroid secretion or failure to correct nutritional deficiency associated to it may have the same effect²⁸⁾. After injection of thyroid hormone, a hyperfunction of hematopoiesis in the bone marrow and an increase in red blood cells were observed. In rabbits thyroidectomized, RHP began to decline gradually

after the operation, while SIC decreased alike. No hyperfunction of hematopoiesis was found in the bone marrow of thyroidectomized rabbits which had been injected with CS by the intravenous route. The hyperfunction of the thyroid tissue in rabbits fed HCD closely resembled that in those intravenously injected with calcium, as was already reported by the authors²³. It is said that a hyperfunction of hematopoiesis in the bone marrow and an increase in number of nucleated cells occur in the condition of hyperthyroid function¹⁷, bringing about an increase of hyperchromic red blood cells in the peripheral venous blood^{2,12}, and that the production of antibody or the resistance to infection is reinforced by the development of the reticuloendothelial system¹³, related closely to erythropoiesis by the utilization of oxygen in tissues by thyroid hormone¹⁰. It is an action of calcium ion in the body to increase the action of adrenalin, and adrenalin stimulates the secretion of thyroid hormone⁴. In consequence, though multiphasic secretion is involved in erythropoiesis¹⁰, the authors have drawn an inference that the thyroid function would play an important part in giving rise to hyperfunction in a series of erythropoietic hematopoiesis in the rabbit fed HCD.

There was a significant difference in serum iron determined this time between the groups fed Ca^{++} supplement and those not fed Ca^{++} supplement. Serum iron in the groups fed Ca^{++} supplement revealed a significantly high value, as compared with that in the groups not fed Ca^{++} supplement. These results were similar to those reported by ANDERSON¹ that a high level of calcium in the diet diminished the formation of insoluble iron phosphates and that excess calcium inhibited iron assimilation. The authors agree with ANDERSON, since they observed that iron phosphates were practically insoluble and, consequently, a high phosphorus content in the diet could interfere with iron absorption unless other factors counteracted this⁷. According to the theory of WHIPPLE²⁷, the test on utilization of food iron, which depends on hemoglobin regeneration, is reliable as a method of measuring iron absorption only when anemia is solely due to iron deficiency. It might be different from the phenomenon that in animals manifesting anemia after injection with carbon tetrachloride, hemoglobin concentration showed a tendency to increase gradually after the feeding of HCD. Nevertheless, the authors believe that the feeding of Ca^{++} supplement in rabbits with liver damage and anemia may be closely related to an increase in hemoglobin and serum iron.

The authors already stated that in healthy animals or those with the liver damaged by intravenous injection of CS, a series of variations were the expression of adaptation reactions of the animal body to the injection as an external stressor. In the experiments reported in this paper, an increase in RHP and lymphocyte count, and the hyperfunction of the bone marrow and thyroid tissues were found. In consequence, the authors presumed that the adaptation reactions of the animals to the feeding or injection of CS would be of the same action mechanism. As reported by the authors in their previous paper²³, probably because the degree of expression by the methods of the reaction in the animal body, the volume administered, and the kinds of calcium used were different, the remarkable variations shown clinically by the animal injected with CS intravenously were not observed in the animal administered with Ca^{++} perorally. Taking the results of all these experiments and the theories mentioned above into consideration, the authors have come to a conclusion that the safety on the body of Ca^{++} , as a stressor, was more excellent in the method of oral administration than in that of intravenous injection.

CONCLUSIONS

In healthy rabbits and dogs administered *per os* with 10 ml of calcium ion solution (CS) per kg of body weight, serum ionic calcium (SIC) showed at first a tendency to decrease momentarily, and then it increased gradually to return to its value before the administration. After peroral administration of 10 ml of CS per kg of body weight daily for 7 days, no

remarkable change was observed in SIC. The red blood count, hemoglobin concentration, and packed-cell volume (RHP) showed a tendency to increase slightly after the first administration. Moreover, a slight hyperfunction was found in the bone marrow and thyroid tissue.

Rabbits with the liver damaged experimentally were divided into 4 groups, SCD, HCD, LCD, and NDD, after the first injection with carbon tetrachloride. Each group was fed a different diet for 4 weeks. In group HCD, fed a diet consisting of Ca^{++} supplement and SCD, or a high calcium diet, there was a slight tendency that RHP and serum iron increased, the bone marrow and thyroid tissue revealed a hyperfunction, and the liver tissue was damaged, as compared with those of group SCD fed a standard calcium diet. The variation in group LCD, fed a low calcium diet, was considered to be brought about by a decrease in RHP, a hypofunction of the bone marrow, and an increase in liver damage, as compared with those of group SCD. On the other hand, of the three rabbits of group NDD, fed a nutritional deficiency diet, two took a fatal course during the period of experiment. Such changes as a decrease in RHP, a hypofunction of the bone marrow and thyroid tissue, and an increase in liver damage were more remarkable in the remaining rabbit of this group NDD than the animal of any other group.

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REFERENCES

- 1) ANDERSON, H. D., McDONOUGH, K. B. and ELVEHJEM, C. A. (1940): *J. Lab. & Clin. Med.*, **25**, 464~471.
- 2) ARNOLD, R. A. and BERMAN, L. (1951): *Blood*, **6**, 436~451.
- 3) BOELTER, M. C. and GREENBERG, D. M. (1941): *J. Nutr.*, **21**, 369~402.
- 4) BOTKIN, A. L. and JENSEN, H. (1952): *Endocrinology*, **50**, 68; cited from ISHII, J. (1959): *Medicine*, **15**, 161~170.
- 5) CANTAROW, A. and TRUMPER, M. (1955): *Clinical Biochemistry*, Philadelphia, W. B. Saunders Co.
- 6) FUKUSHIMA, O. (1954): *Acta Paediat. Jap.*, **59**, 1~4.
- 7) HEGSTEE, D. H., FINCH, C. A. and KINNEY, T. D. (1952): *J. Exp. Med.*, **96**, 115~124.
- 8) HINOHARA, S. (1957): *Water and Electrolytes in Clinical Medicine*, Tokyo, Igaku Shoin Ltd., in Japanese.
- 9) IDA, N., TSUCHIMOTO, I. and HATANO, E. (1954): *Acta Paediat. Jap.*, **58**, 991~993.
- 10) Japan Hematological Society (1963): *Hematology* (2), pp. 474~490, Tokyo, Maruzen Co., in Japanese.
- 11) KATO, K. (1949): *Clinical Haematology*, Tokyo, Bunkodo Co., in Japanese.
- 12) KURIMOTO, T. (1960): *Acta Haemat. Jap.*, **23**, 441~442.
- 13) LURIE, M. B. (1960): *Ann. N. Y. Acad. Sci.*, **88**, 83~98.
- 14) MARTIN, G. J. (1937): *Growth*, **1**, 175~181.
- 15) MARUYAMA, M. (1956): *Acta Paediat. Jap.*, **60**, 241~248.
- 16) MINOT, A. S. and CUTLER, J. T. (1928): *J. Clin. Invest.*, **6**, 369~402.
- 17) MIYAKE, T. and NOTE, S. (1960): *Acta Haemat. Jap.*, **23**, 281~291.
- 18) OKOSHI, S., SUZUKI, N. and TOMODA, I. (1963): *J. Jap. Vet. Med. Ass.*, **16**, 132~136.
- 19) OKOSHI, S., SUZUKI, N. and TOMODA, I. (1963): *J. Jap. Vet. Med. Ass.*, **16**, 210~214.
- 20) OKOSHI, S., SUZUKI, N. and TOMODA, I. (1963): *Jap. J. Vet. Sci.*, **25**, 107~117.
- 21) OKOSHI, S., SUZUKI, N. and TOMODA, I. (1963): *Jap. J. Vet. Sci.*, **25**, 151~163.
- 22) OKOSHI, S., SUZUKI, N. and TOMODA, I. (1963): *Jap. J. Vet. Sci.*, **25**, 299~313.
- 23) OKOSHI, S., and SUZUKI, N. (1965): *Jap. J. Vet. Sci.*, **27**, 1~23.

- 24) QUICK, A. J. (1949): *Amer. J. Physiol.*, **140**, 212~224.
- 25) SHIGEMI, H. (1934): *J. Exp. Gastro-ent.* **9**, 126~142.
- 26) STANBURY, J. B. (1952): *J. Clin. Endocr.* **12**, 191~211.
- 27) WHIPPLE, C. H. and ROBSCHT-ROBBINS, F. S. (1936): *Amer. J. Med. Sci.*, **191**, 11; cited from WINTROBE, M. M. (1962): *Clinical Hematology*, p. 143, Philadelphia, Lea & Febiger.
- 28) WINTROBE, M. M. (1962): *Clinical Hematology*, pp. 460~461, Philadelphia, Lea & Febiger.
- 29) YAMADA, R. (1952): *J. Intern. Med. Jap.*, **40**, 360~368.

家畜血清透析性カルシウムに関する臨床学的研究

VII. 健康および肝障害時における Ca^{++} 経口投与の影響

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健康な家兎および犬に Ca^{++} の経口投与を試みたところ、血清透析性カルシウム (SIC) の血中増加は認められず、経口投与後、一時的に SIC は見かけ上の低下を示し、以後、経口投与前の値に復する動態を観察した。7 日間連続経口投与に際しても、SIC はほとんど変動を示さなかった。赤血球数および血色素濃度は、 Ca^{++} 経口投与後、軽度ながら漸次増加する傾向が認められた。さらに、骨髓および甲状腺の組織は、軽度ではあるが、機能亢進の像を呈していた。

CCl_4 実量 0.01 ml/kg を隔日 3 回、背部に皮下注射した肝障害家兎に、 Ca^{++} 負荷標準 Ca 食 (高 Ca 食)、標準 Ca 食、低 Ca 食および低栄養食を、第 1 回 CCl_4 注射時から 4 週間給与した。標準 Ca 食

給与群に比較して、高 Ca 食給与群では、赤血球数、血色素濃度および血清鉄の増加と、骨髓および甲状腺の機能亢進像を認めた。そしてまた、組織学的検索による肝障害は、わずかではあるが軽減する傾向を示した。低 Ca 食給与群では、標準 Ca 食給与群に比較して、軽度ながら赤血球数および血色素濃度の減少と、骨髓造血能の機能減退を認め、肝障害はやや増悪する傾向を示した。他方、低栄養食給与群では、実験例 3 例のうち、2 例が実験期間中に死の転帰を取った。赤血球数および血色素濃度の減少と、骨髓および甲状腺機能の減退、そして肝障害が増悪する傾向は、実験食給与群全部の中で最も著明であった。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Hepatic cells and Kupffer's stellate cells are normal in regularity. Liver of healthy rabbit administered perorally with Ca^{++} daily for 7 days. Hematoxylin and eosin (H-E) stain. 10×6.3 .
- Fig. 2. The number of small follicles is a little greater than that of large ones in the thyroid tissue. Healthy rabbit administered perorally with Ca^{++} daily for 7 days. H-E stain. 10×6.3 .
- Fig. 3. No remarkable change is found in the marrow tissue, which shows a slight increase of cells. Healthy rabbit administered perorally with Ca^{++} daily for 7 days. H-E stain. 10×10 .
- Fig. 4 and 5. Hepatic cells and Kupffer's cells. Their cytoplasm exhibits almost regular and normal stainability. Group HCD (high calcium diet). H-E stain. 10×6.3 .
- Fig. 6. Small immature follicles are more remarkable than large ones in the thyroid tissue. Group HCD. H-E stain. 10×6.3 .
- Fig. 7. A slight increase of cells showing a hyperfunction in the marrow tissue. Group HCD. H-E stain. 10×10 .
- Fig. 8. Slight infiltration of inflammatory cells is seen. Stainability is almost regular in the liver. Group SCD (standard calcium diet). H-E stain. 10×6.3 .

PLATE II

- Fig. 9. Large and small follicles, almost equal in number, are scattered over the thyroid tissue. Group SCD. H-E stain. 10×6.3 .
- Fig. 10. Hardly any changes are found in the bone marrow. Group SCD. H-E stain. 10×10 .
- Fig. 11. Diversity of nuclear size and slight dilatation of sinusoids are seen in the liver. Group LCD (low calcium diet). H-E stain. 10×6.3 .
- Fig. 12. Colloid in follicles is very light and little in the thyroid tissue. Group LCD. H-E stain. 10×6.3 .
- Fig. 13. Bone marrow showing a slight tendency of cellular hyperplasia. Group LCD. H-E stain. 10×10 .
- Fig. 14. Fatty degeneration of hepatic cells is found diffusely in acini in the liver. Group NDD (nutritional deficiency diet). H-E stain. 10×6.3 .
- Fig. 15. Thyroid tissue composed mostly of large follicles and hardly any small ones. Group NDD. H-E stain. 10×6.3 .
- Fig. 16. Yellow marrow with much fat accompanied by a decrease in cells in the marrow tissue. Group NDD. H-E stain. 10×10 .