CLINICAL STUDIES ON SERUM IONIC CALCIUM IN DOMESTIC ANIMALS

I. SERUM IONIC CALCIUM IN HEALTHY DOGS

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I. SERUM IONIC CALCIUM IN HEALTHY ADULT DOGS AND SOME DOMESTIC ANIMALS

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 through out the body in the form of combination with protein¹²⁾. It has been reported that preserved calcium is found especially in the calcareous tissues¹⁷⁾. Calcium in the body moves constantly between the blood and the bone, and the younger the organism is, the faster it moves. The calcium in the hard tissues has a physiological significance to keep the calcareous density constant in the blood and the tissues. This work is also operated by the serum ionic calcium which is dialytic and active physiologically⁵⁾.

It is thought that the calcium which comes into the body through the mouth is absorbed mainly in the small intestine, especially at its lower level, and takes its course to the portal vein, the liver, the systemic circulation, and the large intestine. Then it is discharged through the small intestine or through the kidney²⁾.

The functional significance of calcium in the body is due to the action of the ionic calcium which is physiologically active. It is related to the acid-base balance in the physical fluid and to the permeation pressure. Calcium in the body has a physiological function to modulate muscular contraction and nervous stimulation by coping with sodium ion and potassium ion. Moreover, it has a close relation to the metabolism of magnesium and phosphorus⁶⁾. The calcium metabolism in the body is strongly related to hormone^{2, 5)} and vitamins^{13, 18)}. Its abnormal phenomena cause various diseases and ailments. In connection with the calcium metabolism, many investigators studied the total calcium in the sera of domestic animals^{3, 6, 7, 15, 16)}. As long as the authors know, no report has been made on the ionic calcium contained in the sera of the domestic animals as yet. There has been no advance in study since 1954 of YANAGISAWA¹⁹⁾, IDA⁸⁾ and HALLGREN³⁾. After careful examination of the dialytic calcium, which was called the ionic calcium, of human serum, MARUYAMA¹⁰⁾ and KATO⁹⁾ found no significant difference between the Yanagisawa method based on a specific color and the McLean method based on a logical calculation from the serum protein and the total calcium.

The present study was carried out to examine the ionic calcium of diseased serum which was thought to have relations with the body defense reaction against various disease factors of the organism suffering from hypocalcemia (low ionic calcium). The total quantity of serum protein was measured and the general state of blood investigated. Then the ratio of the ionic calcium to the total calcium measured as mentined above was obtained. The value of the serum ionic calcium determined by this method was compared with that by the McLean method.

MATERIALS AND METHODS

Materials: Mongrel dogs over 1 year old were used as materials. They had been examined at the veterinary hospital attached to the University of Tokyo and regarded as clinically healthy ones.

Samples of horse, cattle, and swine blood were acquired from the Tokyo Shibaura Slaughterhouse.

The sheep used as materials had been kept in the stock farm attached to the University of Tokyo.

Methods: The dialytic calcium in serum, which is usually called ionic calcium, was measured by Yanagisawa's micro-detection method¹⁹⁾. Generally speaking, the difference between the results of two measurements by this method was regarded as the value of the ionized dialytic calcium. The total calcium was measured by the spectrophotometer. The alkaline liquid of the staining solution Plasmocorin B (1-hydroxy-4-chloro-2,2-diazobenzen-1,8-dihydroxy-naphthalene-3,6-disulfonic acid disodium salt) turned from purple to red when affected by the alkaline-earth metals. The calcium contained was measured as protein-united calcium by making the serum ionic calcium active with saturated ammonium oxalate solution.

A blood sample amounting to 2 ml was drawn from the jugular vein at 10 o'clock in the morning when the animal had an empty stomach. It was so treated as to be measured by the Coleman spectrophotometer at a wave-length of 620 m μ .

To the blood was added a duplicate oxalate solution containing 1.25 mg of ammonium oxalate and 0.75 mg of potassium oxalate per milliliter of blood. Red blood cells were counted by diluting the blood sample 1:100 with Hayem's solution in a red blood cell diluting pipette. White blood cells were counted in a 1:10 dilution of blood in Tuerk solution, by using the hemocytometer of Neubauer. The packed-cell volume was measured by Kato's hemocrit tube and expressed with the value obtained by centrifugation at 3000 rpm for half an hour. Hemoglobin concentration was measured by the acid-hematin electrophotometer method. The total quantity of serum protein was calculated by using the Hitachi serum protein meter. The nomogram of McLean was copied from the textbook of clinical biochemistry by CANTAROW and TRUMPER²⁾. The percentage of each type of leukocytes was calculated from a count of 200 cells.

Samples of horse, cattle, and swine blood were obtained by bloodletting from the heart immediately after death at the Tokyo Shibaura Slaughterhouse.

RESULTS

To see any aberration of observation which would happen from the experimental techniques, the serum ionic calcium and the total calcium were measured five times on one material. The values measured are shown in Table 1. The aberrations of the total calcium were in a range of 0 to 0.8 per cent, the average being 0.6 per cent, and those of the ionic calcium in a range of 0 to 2.4 per cent, the average being 1.2 per cent. Since the aberration limits were within ± 5 per cent, it was assured that there was no error in the experimental techniques.

The values determined on 23 healthy dogs are given Table 2. The average value of the ionic calcium was 4.2 mg per 100 ml (2.1 milliequivalent per liter) and that of the total calcium 11.0 mg per 100 ml (5.5 milliequivalent per liter). The ratio of the ionic calcium to the total calcium was 38.2 per cent. The value of ionic calcium determined by McLean's logical calculation from the total calcium and the total protein was 4.8 mg per 100 ml (2.4 milliequivalent per liter) on the average. Compared with the experimental value, 4.2 mg

Table 1. Values of Serum Ionic Calcium and Total Calcium in Five Materials Collected from a Dog

Material No.	Total Calcium			Protein-bound Ca			Ionic Calcium		
	$\bar{x}$ mg/dl(mEq/l)	$\bar{x}-x$	H*	$\bar{x}$ mg/dl(mEq/l)	$\bar{x}-x$	H*	$\bar{x}$ mg/dl(mEq/l)	$\bar{x}-x$	H*
1	11.4(5.7)	0.1	0.8	7.2(3.6)	0.1	1.4	4.2(2.1)	0	0
2	11.4(5.7)	0.1	0.8	7.1(3.6)	0.2	2.7	4.3(2.2)	0.1	2.4
3	11.5(5.8)	0	0	7.4(3.7)	0.1	1.4	4.1(2.1)	0.1	2.4
4	11.4(5.7)	0.1	0.8	7.2(3.6)	0.1	1.4	4.2(2.1)	0	0
5	11.6(5.8)	0.1	0.8	7.4(3.7)	0.1	1.4	4.2(2.1)	0	0
Average ($\bar{x}$)	11.5(5.8)	0.08	0.6	7.3(3.7)	0.12	1.7	4.2(2.1)	0.04	1.2

Remarks. $H^* = \frac{\bar{x}-x}{\bar{x}} \times 100$

mEq/l = milliequivalent per liter.

Table 2. Means and Distribution of Serum Calcium Content in 23 Healthy Adult Dogs

	Total Ca mg/dl	Ionic Ca mg/dl	McLean Ca mg/dl	Ca ⁺⁺ /T. Ca* %	Total protein g/dl
Mean	11.0	4.2	4.8	38.2	6.8
S.E.**	±0.29	±0.24	±0.23	±3.53	±0.6
Range	9.4—11.8	3.5—5.2	4.4—5.3	30.7—42.5	6.0—7.7

* Total calcium.

** Standard error.

Table 3. Means and Distribution of Blood Values in 23 Dogs Shown in Table 2

	Red count × 10 ⁶	Hb g/dl	Ht %	M.C.H. μg	M.C.V. μ ³	M.C.H.C. %	White count × 10 ³
Mean	6.0	13.2	40	21.8	69.2	31.6	12.3
S.E.	±0.3	±1.7	±3.2	±1.1	±3.1	±1.2	±2.7
	Neutrophils %	Lymphocytes %	Eosinophils %	Monocytes %	Basophils %		
Mean	63.5	19.7	9.8	4.5	0		
S.E.	±3.7	±3.9	±2.2	±3.3			

* Hb=hemoglobin

Ht=hematocrit value

M.C.H.=mean corpuscular hemoglobin

M.C.H.C.=mean corpuscular hemoglobin concentration

M.C.V.=mean corpuscular volume

S.E.=Standard error

Table 4. Means of Serum Calcium Content and Blood Figures in 5 Horses

	Total Ca mg/dl	Ionic Ca mg/dl	McLean Ca mg/dl	Ca ⁺⁺ /T. Ca %	Total protein g/dl
Mean	12.5	5.2	5.6	41.6	7.4
S.E.	±0.28	±0.91	±0.25	±8.39	±0.88
	Red count × 10 ⁶	Hemoglobin g/dl	Hematocrit %	White count × 10 ³	
Mean	8.7	14.3	45.0	6.5	
S.E.	±1.07	±2.33	±4.15	±1.06	

Remarks. For abbreviations see Table 2.

per 100 ml of serum, it was 0.6 mg per 100 ml (0.3 milliequivalent per liter) of serum higher than the latter. The total protein ranged from 6.0 to 7.7 g and averaged 6.8 g per 100 ml of serum. From these values, each confidential boundary could be defined as follows: total calcium, 11.0 ± 0.29 mg; ionic calcium, 4.2 ± 0.24 mg; protein-united calcium, 6.8 ± 0.39 mg; McLean's logical value of ionic calcium, 4.8 ± 0.23 mg; and total protein, 6.8 ± 0.6 g, all per 100 ml of serum.

Table 3 shows blood figures observed on the healthy dogs listed in Table 2. The mean values and distribution were 6.0 ± 0.3 million erythrocytes per cubic millimeter, 12.3 ± 2.7 thousand leukocytes per cubic millimeter, and 13.2 ± 1.7 g of hemoglobin per 100 ml of blood. The packed-cell volume of erythrocytes ranged from 34 to 48 per cent and averaged 40.0 ± 3.2 per cent. The mean and distribution of the mean corpuscular hemoglobin (M.C.H.) was $21.8 \pm 1.1 \mu\mu\text{g}$, that of the mean corpuscular volume (M.C.V.) $69.2 \pm 3.1 \mu^3$, and that of the mean corpuscular hemoglobin concentration (M.C.H.C.) 31.6 ± 1.2 per cent. The mean values of the differential leukocyte count were: neutrophils, 63.5 ± 3.7 per cent; lymphocytes, 19.7 ± 3.9 per cent; eosinophils, 9.8 ± 2.2 per cent; and monocytes, 4.5 ± 3.3 per cent.

Tables 4 to 8 show the results of comparison between the values of serum calcium and blood figures obtained from the 23 healthy adult dogs and the values of serum calcium determined in several other domestic animals.

The mean values and distribution of the serum calcium and blood figures in five horses, as shown in Table 4, were: serum ionic calcium, 5.2 ± 0.91 mg/dl; serum total calcium, 12.5 ± 0.28 mg/dl; and McLean's logical value of ionic calcium, 5.6 ± 0.25 mg/dl. The mean values were 8.7 ± 1.07 million erythrocytes per cubic millimeter, 6.5 ± 1.06 thousand leukocytes per cubic millimeter, and 14.3 ± 2.33 g of hemoglobin per 100 ml of blood. The packed-cell volume of erythrocytes averaged 45.0 ± 4.15 per cent.

In five Holsteins, as shown in Table 5, the mean value of the ionic calcium was 4.2 ± 0.55 mg/dl and that of the total calcium 12.2 ± 0.39 mg/dl. Therefore, the ratio of the former to the latter was 34.4 ± 3.35 per cent. McLean's logical value of ionic calcium was 5.5 ± 0.15 mg/dl. In five Japanese native cows, the average value of the ionic calcium was 4.5 ± 0.71 mg/dl and that of the total calcium 12.3 ± 0.28 mg/dl. Therefore, the ratio of the former to the latter was 37.4 ± 5.68 per cent. McLean's logical value of ionic calcium was 5.5 ± 0.45 mg/dl. In these ten cows, the average value of the ionic calcium was 4.4 ± 0.35 mg/dl and that of the total calcium 12.2 ± 0.18 mg/dl. Therefore, the ratio of the former to the latter was 35.9 ± 2.51 per cent. McLean's logical value was 5.5 ± 0.18 mg per 100 ml.

Table 6 shows the blood picture of the Holstein and the Japanese native cows listed in Table 5. The mean values given by the Holsteins were 7.5 ± 1.4 million erythrocytes per cubic millimeter, 4.9 ± 1.91 thousand leukocytes per cubic millimeter, 12.4 ± 1.38 g of hemoglobin per 100 ml of blood, and 43.0 ± 3.39 per cent packed-cell volume of erythrocytes. The average values given by the Japanese native cows were 9.4 ± 0.71 million erythrocytes, 6.5 ± 1.44 thousand leukocytes, 14.2 ± 1.36 g of hemoglobin, and 50.0 ± 4.03 per cent packed-cell volume of erythrocytes. The average values determined in the cows of both breeds were 8.5 ± 0.61 million erythrocytes, 5.7 ± 0.92 thousand leukocytes, 13.3 ± 0.74 g of hemoglobin, and 46.5 ± 2.02 per cent packed-cell volume of erythrocytes.

As shown in Table 7, five pigs gave the following mean and distribution of the serum calcium and blood figures: serum ionic calcium, 5.1 ± 0.99 mg/dl; serum total calcium, 12.1 ± 1.54 mg/dl; ratio of the former to the latter, 41.8 ± 4.07 per cent; McLean's logical value of ionic calcium, 5.4 ± 0.88 mg/dl; and total protein, 7.0 ± 0.59 g per 100 ml of serum. The mean values in the five pigs were 7.6 ± 1.2 million erythrocytes, 17.1 ± 0.81 thousand leukocytes, 12.6 ± 1.59 g of hemoglobin, and 44.0 ± 6.52 per cent packed-cell volume

Table 5. Means and Distribution of Serum Calcium Content in 10 Cattle

Cattle	Total Ca mg/dl	Ionic Ca mg/dl	McLean Ca mg/dl	Ca ⁺⁺ /T. Ca %	Total protein g/dl
HOLSTEIN					
Mean	12.2	4.2	5.5	34.4	6.8
S.E.	±0.39	±0.55	±0.15	±3.35	±0.14
JAPANESE NATIVE					
Mean	12.3	4.5	5.5	37.4	7.1
S.E.	±0.28	±0.71	±0.45	±5.68	±0.96
CATTLE					
Mean	12.2	4.4	5.5	35.9	6.9
S.E.	±0.18	±0.35	±0.18	±2.51	±0.37

Table 6. Means and Distribution of Blood Figures in 10 Cattle Shown in Table 5

Cattle	Red count × 10 ⁶	Hemoglobin g/dl	Hematocrit %	White count × 10 ³
HOLSTEIN				
Mean and S.E.	7.5±1.40	12.4±1.38	43.0±3.39	4.9±1.91
JAPANESE NATIVE				
Mean and S.E.	9.4±0.71	14.2±1.36	50.0±4.03	6.5±1.44
CATTLE				
Mean and S.E.	8.5±0.61	13.3±0.74	46.5±2.02	5.7±0.92

Table 7. Means of Serum Calcium and Blood Figures in 5 Figs

	Total Ca mg/dl	Ionic Ca mg/dl	McLean Ca mg/dl	Ca ⁺⁺ /T. Ca %	Total protein g/dl
Mean	12.1	5.1	5.4	41.8	7.0
S.E.	±1.54	±0.99	±0.88	±4.07	±0.59
	Red count × 10 ⁶	Hemoglobin g/dl	Hematocrit %	White count × 10 ³	
Mean and S.E.	7.6±1.21	12.6±1.59	44.0±6.52	17.1±0.81	

Table 8. Means of Serum Calcium Content and Blood Figures in 10 Sheep

	Total Ca mg/dl	Ionic Ca mg/dl	McLean Ca mg/dl	Ca ⁺⁺ /T. Ca %	Total protein g/dl
Mean	11.0	4.4	5.3	40.1	5.9
S.E.	±0.24	±0.29	±0.21	±2.21	±0.44
	Red count × 10 ⁶	Hemoglobin g/dl	Hematocrit %	White count × 10 ³	
Mean and S.E.	11.5±1.64	12.4±1.42	29.0±3.98	9.2±3.21	

of erythrocytes.

In ten sheep shown in Table 8, the mean and distribution of the serum calcium and blood picture were as follows: serum ionic calcium, 4.4±0.29 mg/dl; serum total calcium, 11.0±0.24 mg/dl; ratio of the former to the latter, 40.1±2.21 per cent; McLean's logical value of ionic calcium, 5.3±0.21 mg/dl; total protein, 5.9±0.44 g per 100 ml of serum;

Table 9. Comparison of Serum Calcium among Healthy Adult Dogs, Horses, Cattle, Pigs, and Sheep

Species	Ionic Calcium mg/dl	Total Calcium mg/dl	Ca ⁺⁺ /T. Ca %
Dog	4.2(0)	11.0(0)	36.5(0)
Horse	5.2(+1.0)	12.5(+1.5)	41.6(+5.1)
Cattle	4.4(+0.2)	12.2(+1.2)	35.9(-0.6)
Pig	5.1(+0.9)	12.1(+1.1)	41.8(+5.3)
Sheep	4.4(+0.2)	11.1(+0.1)	40.1(+3.6)

Fig. 1. Means and Standard Errors of Serum Calcium Content in Dogs at Various Ages

and serum calcium is more useful for the veterinary field than mere averages in dogs at various ages. The present paper presents such information obtained from 87 dogs from 24 hours to over 2 years of age.

This work was carried out to provide veterinarians in the dog practice with information on the serum calcium, especially the ionic calcium, and blood values in the physiological range of the dog.

METHODS

The dogs under 15 weeks of age were born from apparently healthy bitches at the veterinary hospital attached to the University of Tokyo. They had been given routine care and feed as laboratory dogs.

Blood samples, 1.5 ml each in quantity, were collected under as nearly identical conditions as possible with respect to the time of the day, time intervals between feeding and bleeding, and the degree of excitement. Bleeding was made from the jugular vein into a bleeding tube containing the duplicate oxalate in all animals, except those 24 hours of age. A drop of blood remaining in the bleeding needle was placed on a clean glass slide, so that a differential film might be prepared. In animals 24 hours of age, blood samples were collected directly from the heart by bloodletting.

The methods mentioned above in Chapter I were used for measurement.

11.5±1.64 million erythrocytes; 9.2±3.21 thousand leukocytes; 12.4±1.42 g of hemoglobin per 100 ml of blood, and 29.0±3.98 per cent packed-cell volume of erythrocytes.

In Table 9, the calcium in the serum was compared among horses, cattle, pigs, sheep, and healthy adult dogs. The values of the total calcium were determined in horses, cattle, pigs, sheep, and dogs, those of the ionic calcium in horses, pigs, cattle, sheep, and dogs on and of pigs, horses, sheep, dogs, and cattle on the ratio of the later to the former.

II. SERUM IONIC CALCIUM OF HEALTHY DOGS AT VARIOUS AGES

Information on the degree of variation in the means and distributions of blood values

Table 10. Means of Serum Calcium and Blood Figures in Dogs at Various Ages

Age	No. of dogs	24 hrs.	1 week	3 wks.	5 wks.	10 wks.	15 wks.	1 year	over 2 yrs.
		5	5	15	17	17	10	9	15
SERUM		Mean $\pm$ Standard error							
Calcium	mg/dl								
Ionic		4.0 $\pm$ 0.8	3.7 $\pm$ 0.5	4.4 $\pm$ 0.3	4.3 $\pm$ 0.2	4.5 $\pm$ 0.3	3.8 $\pm$ 0.2	4.1 $\pm$ 0.4	4.3 $\pm$ 0.3
Total		9.5 $\pm$ 0.6	10.3 $\pm$ 1.1	10.3 $\pm$ 0.4	10.0 $\pm$ 0.3	10.6 $\pm$ 0.4	10.5 $\pm$ 0.4	10.7 $\pm$ 0.5	11.1 $\pm$ 0.3
McLean		5.0 $\pm$ 0.4	4.7 $\pm$ 1.1	5.5 $\pm$ 0.3	5.5 $\pm$ 0.3	5.4 $\pm$ 0.3	5.1 $\pm$ 0.4	4.8 $\pm$ 0.3	4.9 $\pm$ 0.3
Ca ⁺⁺ /T. Ca %	%	42.4 $\pm$ 4.8	35.9 $\pm$ 5.4	42.7 $\pm$ 3.2	43.0 $\pm$ 3.1	42.3 $\pm$ 4.4	38.4 $\pm$ 4.2	38.3 $\pm$ 4.1	39.1 $\pm$ 3.9
Protein	g/dl								
Total		4.7 $\pm$ 0.6	4.7 $\pm$ 0.2	4.9 $\pm$ 0.6	4.6 $\pm$ 0.3	4.8 $\pm$ 0.5	5.3 $\pm$ 0.8	6.7 $\pm$ 0.7	7.0 $\pm$ 0.7
BLOOD									
R.B.C.	$\times 10^6$	5.3 $\pm$ 0.4	4.0 $\pm$ 0.3	4.1 $\pm$ 0.2	3.4 $\pm$ 0.7	4.8 $\pm$ 0.1	4.7 $\pm$ 0.2	6.2 $\pm$ 0.5	5.8 $\pm$ 0.4
Hb.	g/dl	14.1 $\pm$ 1.2	10.7 $\pm$ 0.9	9.2 $\pm$ 2.8	7.8 $\pm$ 1.3	8.9 $\pm$ 0.7	8.6 $\pm$ 1.4	13.8 $\pm$ 1.9	12.5 $\pm$ 1.3
Ht.	%	54.4 $\pm$ 4.8	36.0 $\pm$ 3.8	29.0 $\pm$ 5.3	25.0 $\pm$ 4.6	34.0 $\pm$ 3.2	34.1 $\pm$ 3.1	43.3 $\pm$ 4.6	39.1 $\pm$ 3.8
W.B.C.	$\times 10^3$	9.7 $\pm$ 3.1	7.7 $\pm$ 2.7	11.1 $\pm$ 1.4	13.6 $\pm$ 4.3	12.4 $\pm$ 3.9	16.8 $\pm$ 4.1	10.8 $\pm$ 3.1	12.9 $\pm$ 3.0
Neut.	%	65.1 $\pm$ 2.9	48.0 $\pm$ 7.3	79.3 $\pm$ 4.1	51.2 $\pm$ 8.1	65.5 $\pm$ 4.4	70.1 $\pm$ 4.1	64.8 $\pm$ 4.3	62.5 $\pm$ 4.8
Lymph.	%	31.6 $\pm$ 4.2	37.3 $\pm$ 6.1	20.4 $\pm$ 5.6	45.1 $\pm$ 3.8	27.3 $\pm$ 2.3	24.8 $\pm$ 3.9	18.2 $\pm$ 5.1	24.6 $\pm$ 2.9
Eosin.	%	3.6 $\pm$ 2.9	14.1 $\pm$ 5.8	1.1 $\pm$ 0.9	3.3 $\pm$ 2.7	7.9 $\pm$ 3.6	6.4 $\pm$ 2.7	10.1 $\pm$ 3.2	9.3 $\pm$ 2.1
Mono.	%	0.2 $\pm$ 0.5	1.1 $\pm$ 0.8	0	1.3 $\pm$ 0.4	0.3 $\pm$ 0.2	1.4 $\pm$ 0.8	7.9 $\pm$ 3.8	3.5 $\pm$ 2.7

Remarks. For abbreviations see Tables 2 and 3.

RESULTS

The means and standard errors for each of the eight groups are given in Table 10 and Fig. 1.

The average values in a group of five 24-hour-old animals were as follows: serum ionic calcium, 4.0 ± 0.8 mg/dl; serum total calcium, 9.5 ± 0.6 mg/dl; ratio of the former to the latter, 42.4 ± 4.8 per cent; McLean's ionic calcium, 5.0 ± 0.4 mg/dl; serum total protein, 4.7 ± 0.6 g per 100 ml; 5.3 ± 0.4 million erythrocytes and 9.7 ± 3.1 thousand leukocytes per cubic millimeter; 14.1 ± 1.2 g of hemoglobin per 100 ml of blood; 54.4 ± 4.8 per cent packed-cell volume of erythrocytes; 65.1 ± 2.9 per cent neutrophils, 31.6 ± 4.2 per cent lymphocytes, 3.6 ± 2.9 per cent eosinophils, and 0.2 ± 0.5 per cent monocytes in the differential leukocyte count.

The average values in a group of five 1-week-old animals were as follows: serum ionic calcium, 3.7 ± 0.5 mg/dl; serum total calcium, 10.3 ± 1.1 mg/dl; ratio of the former to the latter, 35.9 ± 5.4 per cent; McLean's ionic calcium, 4.7 ± 1.1 mg/dl; total protein, 4.7 ± 0.2 g per 100 ml of serum; 4.0 ± 0.3 million erythrocytes and 7.7 ± 2.7 thousand leukocytes per cubic millimeter, 10.7 ± 0.9 g of hemoglobin per 100 ml of blood, 36.0 ± 3.8 per cent packed-cell volume of erythrocytes; 48.0 ± 7.3 per cent neutrophils, 37.3 ± 6.1 per cent lymphocytes, 14.1 ± 5.8 per cent eosinophils, and 1.1 ± 0.8 per cent monocytes in the differential leukocyte count.

The average values in a group of fifteen 3-week-old animals were as follows: serum ionic calcium, 4.4 ± 0.3 mg/dl; serum total calcium, 10.3 ± 0.4 mg/dl; ratio of the former to the latter, 42.7 ± 3.2 per cent; McLean's ionic calcium, 5.5 ± 0.3 mg/dl; total protein, 4.9 ± 0.6 g per 100 ml of serum; 4.1 ± 0.2 million erythrocytes and 11.1 ± 1.4 thousand leukocytes per cubic millimeter, 9.2 ± 2.8 g of hemoglobin per 100 ml of blood, 29.0 ± 5.3 per cent packed-cell volume of erythrocytes; 79.3 ± 4.1 per cent neutrophils, 20.4 ± 5.6 per cent lymphocytes, and 1.1 ± 0.9 per cent eosinophils in the differential leukocyte count.

The average values in a group of seventeen 5-week-old animals were as follows: serum ionic calcium, 4.3 ± 0.2 mg/dl; serum total calcium, 10.0 ± 0.3 mg/dl; ratio of the former to the latter, 43.0 ± 3.1 per cent; McLean's ionic calcium, 5.5 ± 0.3 mg/dl; total protein 4.6 ± 0.3 g per 100 ml of serum; 3.4 ± 0.7 million erythrocytes and 13.6 ± 4.3 thousand leukocytes per cubic millimeter, 7.8 ± 1.3 g of hemoglobin per 100 ml of blood, 25.0 ± 5.3 per cent packed-cell volume of erythrocytes; 51.2 ± 8.1 per cent neutrophils, 45.1 ± 3.8 per cent lymphocytes, 3.3 ± 2.7 per cent eosinophils, and 1.3 ± 0.4 per cent monocytes in the differential leukocyte count.

The average values in a group of eleven 10-week-old animals were as follows: serum ionic calcium, 4.5 ± 0.3 mg/dl; serum total calcium, 10.6 ± 0.4 mg/dl; ratio of the former to the latter, 42.3 ± 4.4 per cent; McLean's ionic calcium, 5.4 ± 0.3 mg/dl; total protein, 4.8 ± 0.5 g per 100 ml of serum; 4.8 ± 0.1 million erythrocytes and 12.4 ± 3.9 thousand leukocytes per cubic millimeter, 8.9 ± 0.7 g of hemoglobin per 100 ml of blood, 34.0 ± 3.2 per cent packed-cell volume of erythrocytes; 65.5 ± 4.4 per cent neutrophils, 27.3 ± 2.3 per cent lymphocytes, 7.9 ± 3.6 per cent eosinophils, and 0.3 ± 0.2 per cent monocytes in the differential leukocyte count.

The average values in a group of ten 15-week-old animals were as follows: serum ionic calcium, 3.8 ± 0.2 mg/dl; serum total calcium, 10.5 ± 0.4 mg/dl; ratio of the former to the latter, 38.4 ± 4.2 per cent; McLean's ionic calcium, 5.1 ± 0.4 mg/dl; total protein, 5.3 ± 0.8 g per 100 ml of serum; 4.7 ± 0.2 million erythrocytes and 16.8 ± 4.1 thousand leukocytes per cubic millimeter, 8.6 ± 1.4 g of hemoglobin per 100 ml of blood, 34.1 ± 3.1 per cent packed-cell volume of erythrocytes; 70.1 ± 4.1 per cent neutrophils, 24.8 ± 3.9 per cent lymphocytes, 6.4 ± 2.7 per cent eosinophils, and 1.4 ± 0.8 per cent monocytes in the differential

leukocyte count.

The average values in a group of nine 1-year-old animals were as follows: serum ionic calcium, 4.1 ± 0.4 mg/dl; serum total calcium, 10.7 ± 0.5 mg/dl; ratio of the former to the latter, 38.3 ± 4.1 per cent; McLean's ionic calcium, 4.8 ± 0.3 mg/dl; total protein, 6.7 ± 0.7 g per 100 ml of serum; 6.2 ± 0.5 million erythrocytes and 10.8 ± 3.1 thousand leukocytes per cubic millimeter, 13.8 ± 1.9 g of hemoglobin per 100 ml of blood, 43.3 ± 4.6 per cent packed-cell volume of erythrocytes; 64.8 ± 4.3 per cent neutrophils, 18.2 ± 5.1 per cent lymphocytes, 10.1 ± 3.2 per cent eosinophils, and 7.9 ± 3.8 per cent monocytes in the differential leukocyte count.

The average values in a group of fifteen over-2-year old animals were as follows: serum ionic calcium, 4.3 ± 0.3 mg/dl; serum total calcium, 11.1 ± 0.3 mg/dl; ratio of the former to the latter, 39.1 ± 3.9 per cent; McLean's ionic calcium, 4.9 ± 0.3 mg/dl; total protein, 7.0 ± 0.7 g per 100 ml of serum; 5.8 ± 0.4 million erythrocytes and 12.9 ± 3.0 thousand leukocytes per cubic millimeter, 12.5 ± 1.3 g of hemoglobin per 100 ml of blood, 39.1 ± 3.8 per cent packed-cell volume of erythrocytes; 62.5 ± 4.8 per cent neutrophils, 24.6 ± 2.9 per cent lymphocytes, 9.3 ± 2.1 per cent eosinophils, and 3.5 ± 2.7 per cent monocytes in the differential leukocyte count.

III. DISCUSSION

From the results above, the authors calculated the mean values of ionic calcium and total calcium to be 4.2 mg/dl (2.1 mEq/l) and 11.0 mg/dl (5.5 mEq/l). These values nearly coincided with those given by the previous workers^{8,19} and were very close to McLean's logical values of ionic calcium. Taking this fact into consideration, the authors suppose that the values determined by the Yanagisawa method are quite reliable.

It is said that there are three types of serum calcium. The first type is combined mainly with serum protein, which does not pass through a dialytic film, and the type is inactive calcium. The second type is ionized and physiologically active calcium, or ionized dialytic calcium. The third type is non-ionized dialytic calcium preserved in the form of salt. The second and third types are dialytic calcium and pass through a dialytic film. Of the three types of calcium, the second type, or ionized dialytic calcium, has direct relations to the metabolism of inorganic matter and to the physiological function of the body²¹. Non-ionized dialytic calcium is very small in quantity in the serum, being under 0.6 mg per 100 ml, as reported by McLEAN¹¹. The authors call non-ionized and ionized dialytic calcium *ionic calcium*. The ratio of dialytic calcium to total calcium is of almost constant value in the quite healthy animal. Dialytic calcium keeps balance with the other inorganic cations when it is among such anions Cl, HPO_4 , and HCO_3 , acids, and bases¹⁷.

The dogs studied gave a mean value of 0.6 mg/dl (0.3 mEq/l), the pigs and horses 0.4 mg/dl (0.2 mEq/l), the cattle 1.1 mg/dl (0.55 mEq/l), and the sheep 0.9 mg/dl (0.45 mg/l).

These values were the differences between the actually measured quantities and McLean's theoretical values of serum ionic calcium in the healthy animals, the latter being greater than the former. This fact cannot be explained only with the extent of the error caused by the experimental techniques. As McLean and Hasting's method of calculation is based on the hypothesis that the combination of calcium ion and serum protein is reversible, it must be carried out on the condition that reversible balance is present in the blood plasma and that samples are derived from quite healthy normal animals. If these conditions are not satisfied, or if changes occur in serum protein after the primary or secondary disease, it is presumed that no difference between McLean's theoretical value and the actually measured value is useful and, moreover, that at the time of disease when any changes occur in serum protein, McLean's theoretical values are inapplicable. For these

reasons, it is unavoidable that there is any difference between the theoretical value and the actually measured value in domestic animals.

The authors could find no significant differences in the total calcium and ionic calcium of the serum among eight different age groups of healthy puppies and adult dogs. A slight decrease in the total and ionic calcium was observed in two groups of 1- and 15-week-old animals. A low average value of total and ionic calcium was obtained from the 1-week-old puppies, because they were in the rapidly growing stage and had less serum calcium than the lactating bitch. The serum ionic calcium was less in the 15-week-old dogs than in the 1-week-old, even when the former had been fed a supplemental ration consisting of fresh fish, meat, soft food, and powdered milk and containing equilibrated lactose and vitamins, or a commercial supplement. No definite conclusions would be drawn from this experiment, since any conditions causing the development of such hard tissues as bone and tooth have been unknown. In the case of the 15-week-old dogs which were in the early stage of adolescence, it is doubtful that the development in physical conditions alone had great influence on the serum calcium and blood values observed. It would appear neither nutritional level for the lactating bitches nor supplemental ration for the puppies was sufficient to satisfy calcium intake and/or utilization.

The data presented in this paper indicate that physiological fluctuation in serum calcium and blood pictures will take place in dogs of various ages, from 24-hour-old to adult ones.

IV. SUMMARY

In 23 healthy adult dogs examined for serum calcium, ionic calcium was 4.2 ± 0.24 mg/dl, total calcium 11.0 ± 0.29 mg/dl, and the ratio of ionic to total calcium 38.2 ± 3.53 per cent.

The serum ionic calcium was 5.2, 4.4, 5.1, and 4.4 mg/dl in horses, cattle, pigs, and sheep, respectively. It was higher in these domestic animals than in the dogs.

In the dogs studied, no agreement was observed between the actually measured value and McLean's theoretical value of the serum ionic calcium. The latter value was higher than the former, although both values were comparatively close to each other.

There were no significant differences in the serum calcium among eight different age groups of healthy puppies and adult dogs. A slight decrease in serum ionic calcium was observed in two groups consisting of one- and fifteen-week-old dogs, respectively.

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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

I. 健康犬の血清透析性カルシウム

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カルシウムの生体内作用は、イオン化 Ca の作用によると考えられている。そこで著者らは、家畜内科学の面から、Ca に関係があると考えられる疾病の血清透析性イオン化 Ca を検討し、これが変動するとき、とくに低血清透析性 Ca 状態が生体の防御反応に対する意義を究明しようとした。

第 I 報においては、健康犬血清 Ca の年齢的動揺範囲とともに、血清総蛋白量および血液の一般性状を測定した。ついで、これによって得た総 Ca に対する透析性 Ca の比率 (イオン化率) を求め、血清透析性 Ca (SIC と略す) について、柳沢法による実測値と McLEAN の理論値 (それぞれ単に実測値、理論値と呼ぶ) とを比較検討した。

健康成犬 23 例について、血清 Ca を測定した。その結果、平均値をとると、SIC は 4.2 ± 0.24 mg/dl (以下単位をはぶく)、血清総 Ca は 11.0 ± 0.29 、蛋白結合型 Ca は 6.8 ± 0.39 となり、理論値から求めた血清イオン化 Ca の平均は 4.8 ± 0.23 であった。

馬 5 例の平均では、SIC が 5.2 ± 0.91 、理論値が 5.6 ± 0.25 、血清総 Ca が 12.5 ± 0.28 であった。

牛 10 例の平均では、SIC が 4.4 ± 0.35 、理論値が 5.5 ± 0.18 、血清総 Ca が 12.2 ± 0.18 であった。

豚 5 例の平均では、SIC が 5.1 ± 0.99 、理論値が

5.4 ± 0.88 、血清総 Ca が 12.1 ± 1.54 であった。

羊 10 例の平均では、SIC が 4.4 ± 0.29 、理論値が 5.3 ± 0.21 、血清総 Ca が 11.0 ± 0.24 であった。

健康と見なされた犬 87 例を、年齢的変動を観察する目的で、生後 24 時間・1 週令・3 週令・5 週令・15 週令・1 才および 2 才以上の 8 群に分け、血清 Ca を測定した。その結果、1 週令群および 15 週令群において、SIC が低下する傾向を認めた。

これらの測定成績をみると、健康成犬を始め、馬・牛・豚・羊において、いずれも理論値が実測値よりも高い。このことは、実験上の手技の誤差範囲だけでは、説明できないものがある。

McLEAN の計算は、Ca イオンと蛋白質の結合が血漿内において可逆平衡の状態にあるという仮説のもとに作成された式および図表を用いている。そこで、動物は全く健康かつ正常であることを前提としているはずである。したがって、この前提がくずれた場合には、理論値が実測値と異なることがあり得ると考えられる。以上の理由から、ここに得られた実測値と理論値との差は、いたし方ないものと思われる。今後は、各種の内科疾患における血清 Ca の動態を検索するとともに、その異常状態が生体に及ぼす影響を追究していきたい。