Pathophysiological Study on Caval Syndrome of Dirofilariasis in Dogs

犬糸状虫症 caval syndrome における病態生理学的研究

北川均

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岐阜大学農学部

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CONTENTS

I. PREFACE

Caval syndrome (CS) is a type of canine dirofilariasis. The disease has been described by various synonyms such as dirofilarial hemoglobinuria, venae cavae syndrome, venae cavae embolism, post-caval syndrome, liver failure syndrome or acute hepatic syndrome [6, 17, 25, 30]. Caval syndrome has occurred in a small part of heartworm-infected dogs (3.9 % in Japan [28] and 10-15 % in Australia [5]). However, it has been considered that the syndrome was one of the important diseases in veterinary clinical medicine, because of acute and violent symptoms and high mortality. Dogs with CS develop suddenly characteristic symptoms such as anorexia, depression, dyspnea, anemia, systolic cardiac murmur, jugular pulse and hemoglobinuria. These signs are complicated by severe liver and renal failure in many dogs. Most dogs die on acute course, if they do not receive an adequate treatments; surgical heartworm removal with venotomy and palliative internal medications.

This disease was reported first in the U.S.A. by Adams [2] in 1956. He described a case of dirofilariasis with the signs of CS, in which heartworms were located in the pulmonary arteries, right ventricle, right atrium, posterior vena cava and hepatic veins. Jackson et al. [31] and Lichtenberg et al. [41] reported clinical findings, location of heartworms in autopsied cases and the gross and microscopic observations, and they termed the disease "venae cavae syndrome" from the location of heartworms. They also used the term "liver failure

syndrome" for the disease from the findings of severe liver injuries [30]. In 1963, the condition was induced by experimental infections of a large number of 3rd-stage larvae [48]. Jackson et al. [32] again reported the distribution of heartworms in the right side of the heart and adjacent vessels in autopsied dogs in 1966. In Japan, the study started by Fujii [17] from the surgical treatment with venotomy using a straight and rigid alligator forceps along with clinical observations in dogs with the disease. Almost the same surgical method of venotomy has been reported in the U.S.A. by Jackson et al. [33]. More recently, surgical treatment using a flexible alligator forceps, which can remove successfully the worms also from the pulmonary arteries, has been reported by the author and colleagues in 1985 [27].

Histopathological findings in dogs with CS were described by Lichtenberg et al. [41] and Nomura et al. [43]. The clinical pathology was reported again by Atwell and Boreham [7], and possible mechanisms of CS have been suggested in Australia [5]. Recently, suggestive studies for heartworm migration by echocardiographic findings of the left heart have been undertaken in the U.S.A. by Atkins et al. [3, 4]. Classification of CS by two-dimensional echocardiography was also attempted [47]. However, there has been no systemic studies on the the pathophysiology of the disease including mechanism of intravascular hemolysis. On heartworm migration, some investigators proposed that the disease was induced by the parasitism of a large number of heartworms in the

pulmonary arteries [33, 48]. On intravascular hemolysis, the mechanism of disseminated intravascular coagulation, in which erythrocytes were destroyed physically with fibrin strand in the peripheral vascular system, was proposed [40]. However, these proportions on heartwrom migration and intravascular hemolysis could not explain the pathophysiology of the disease, because the dogs harboring a small number of heartworms were also affected by the syndrome, and almost all dogs failed to show bleeding diathesis and abnormal blood coagulation.

Different from pulmonary heartworm disease, in which heartworms locate only in the pulmonary arteries, dogs with CS harbored haertworms in the pulmonary arteries, right ventricle, right atrium and venae cavae. Some investigators suggested that the habitat of heartworms playing the most important role in pathophysiology of the disease was the venae cavae [31] or right atrium [10]. However, clinical signs such as positive jugular pulsation and systolic cardiac murmur indicate tricuspid valve dysfunction. Insertion of heartworm-like electric wire into the venae cavae could not reproduce the symptoms of CS [42]. Therefore, the location of heartworms associated closely with pathophysiology of CS has not been established. In spite of severe signs indicating circulatory disturbance, hemodynamics have not been determined in dogs with CS. Then, relationships between the location of heartworms and circulatory disturbance or increase in intravascular hemolysis have been unknown.

In SECTION I, in order to clarify the relationship between the location of heartworms and pathophysiology of the disease, parasitism site of heartworms was investigated in dogs with naturally acquired CS, and cardiopulmonary function values were measured before and after surgical heartworm removal in dogs with spontaneous CS. The values were also measured in dogs with artificial model of CS.

In SECTION II, in order to probe the mechanism of intravascular hemolysis, the study describes relationship between the location of heartworms and plasma hemoglobin concentration, findings of plasma and erythrocyte membrane lipid contents, erythrocyte fragilities, circulating erythrocyte shapes and other possible factors inducing intravascular hemolysis.

In SECTION III, the study investigates the cause of heartworm migration from the pulmonary arteries towards the right atrium. The onset of CS is occurrence of heartworms at the tricuspid valve orifice, that is migration of heartworms from the pulmonary arteries. The author attempts to change hemodynamics of dogs harboring heartworms only in the pulmonary arteries, and to identify the factors inducing heartworm migration.

2. SECTION I. Parasitic location of heartworms and right heart hemodynamics

Introduction

Dogs with CS may reveal various symptoms following circulatory disturbance, intravascular hemolysis, anemia, renal failure and/or hepatic dysfunction [3, 6, 7, 17, 25, 31, 33, 45]. The symptoms are known to be relieved after surgical removal of heartworms by venotomy in most dogs [17, 26, 33], and it has been considered that the heartworms must be cause to the occurrence of the disease. However, the location of heartworms to associate primarily with the occurence of the disease has not been established. Until now, the location of heartworms had been examined by autopsy. However, it was recognized that heartworms had migrated after death of the dog [20]; the worms upon autopsy were no longer found in the original habitat. Recently, two-dimensional echocardiography has been employed in veterinary clinical medicine [19, 57]. The location of heartworms can be observed in real time, and it is possible to investigate the relationship between the location of heartworms and the disease. In spite of symptoms following severe circulatory disturbance, cardiopulmonary function values in dogs with this disease has not been investigated. Thus, where and why heartworms cause disturbance in circulation and development of symptoms have yet be explained.

In this section, in order to investigate the pathophysiology of CS, location of adult heartworms in the right heart system is examined from observations of echocardiography, results of surgical removal with flexible

alligator forceps and autopsied findings. Then, right heart hemodynamics are determined in dogs with spontaneous CS before and after surgical heartworm removal using a flexible or straight alligator forceps. Moreover, heartworm-like silicone tubes were inserted into the right atrium and tricuspid valve orifice. The author tries to determine whether the same symptoms and hemodynamics are reproduced as in spontaneous cases.

Materials and methods

A total of 54 dogs with spontaneous CS were used. Dogs were outpatients of the Veterinary Hospital, Faculty of Agriculture, Gifu University, or were introduced from regional dog pounds. The disease was diagnosed on the basis of clinical signs, laboratory test results and echocardiographic findings. Precise histories of dogs were unknown, and periods from onset to diagnosis were various.

Heartworm removal from the right atrium and tricuspid valve orifice through the jugular vein was performed by the modified Fujii method [17] by use of the straight alligator forceps or by use of the flexible alligator forceps [27]. Heartworms were removed only from the right atrium by use of a straight forceps, and also from the pulmonary arteries by use of a flexible alligator forceps.

Location of heartworms was observed by two-dimensional echocardiography (EUB-40 Ultrasonic Convex Array Scanner, Hitachi Medical Corp., Tokyo). Right-lateral and dorso-ventral

thoracic radiographs were obtained from each dog. On angiocardiography, as a contrast medium, about 60 % metholizoacid (Isopaque, Torii & Co. Ltd., Tokyo) was infused through an NIH-catheter (6.5 F, Cook Inc., Bloomington, Indiana) into the right ventricle.

Central venous pressure (CVP) was determined with a fluid manometer (C.V.P. Set, Top Surgical Mfg. Co. Ltd., Tokyo) connected to a catheter filled with 3.8 % sodium citrate solution. Dogs was restrained in right lateral recumbency, and anesthetized locally with procaine hydrochloride. The height of 0-reference was arranged so as to correspond to the midpoint of thoracic thickness at the 3rd intercostal space. CVP determination was carried out immediately before and after surgical heartworm removal through the jugular vein with a straight forceps.

Cardiopulmonary function measurements were carried out in dogs undergoing surgical heartworm removal using a flexible alligator forceps. For measurements of blood pressures in the right heart and right cardiac output, dogs were anesthetized generally by intramuscular injection of 0.25-0.5 mg/kg body weight of diazepam (Cercine, Takeda Chemical Industries Ltd., Osaka) and 2.0-5.0 mg/kg body weight of ketamine hydrochrolide (Ketalar, Sankyo Co. Ltd., Tokyo). The same posture and 0-references as the measurement of CVP were used for measurements of pressures of the pulmonary artery, right ventricle and right atrium. Pressures were measured through a fluid-filled catheter connected with a blood pressure

transducer (TP-101T, Nihon Kohden Corp., Tokyo) and multipurpose polygraph (RM-85, Nihon Kohden Corp.). Right cardiac output was determined with a Swan-Ganz flow-directed pediatric thermodilution catheter (93-132-5F, Edwards Lab., Santa Ana, California, U.S.A.) and computers for measurement of cardiac output (AH-611V and EQ-611V, Nihon Kohden Corp.). Cardiac index (cardiac output/kg body weight of the dog) and stroke volume (cardiac output/heart rate), total pulmonary resistance (mean pulmonary arterial pressure (mmHg) x 1332 x 60/cardiac index (ml/min/kg)) and right ventricular stroke work index (stroke index (ml/beat/kg) x mean pulmonary arterial pressure (mmHg) x 0.0136 [12]) were calculated from each parameter. Pressure and cardiac output measurements were carried out before heartwrom removal and 1 and 7 days after the treatment.

In 6 dogs, heartworm-like silicone tubes (M-Silico, No. 00, Shin-Etsu Polymer Co. Ltd., Tokyo), 0.5-1.0 mm in diameter, 250 mm in length, having a heartworm-like flexibility and softness and containing the contrast medium for angiocardiography (Isopaque 440, Torii & Co. Ltd., Tokyo), were inserted into the tricuspid valve orifice and right atrium using the flexible alligator forceps. Three dogs were heartworm-free, and other 3 dogs were heartworm-infected. The infected dogs were used for the experiment after removal of heartworms from the pulmonary arteries. The number of tubes inserted was from 7 to 12 (small-number group), and from 15 to 32 (large-number group). Initially, most tubes had flew out to

the pulmonary arteries. However, after being returned to the tricuspid valve orifice from the pulmonary arteries, the tubes stayed at the tricuspid valve orifice and in the right atrium. After insertion of the tubes, clinical signs of the dogs were observed carefully. Right heart hemodynamics were determined before and 1 and 7 days after insertion with the same procedures mentioned above.

Results

1) Location of live heartworms in the right heart

On echocardiography (Figs. I-1 to 6), spotted filarid worm echoes were observed at the tricuspid valve orifice in all dogs with CS. the echogenic and spotted mass of heartworms

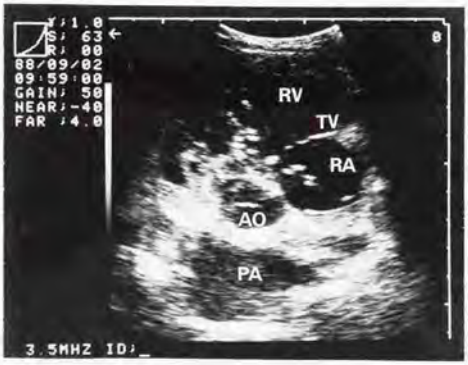

Fig. I-1. B-mode echocardiogram at the diastolic phase in a spontaneous case. Heartworm echoes locates at the tricuspid valve orifice. RV: Right ventricle, TV: Tricuspid valve, RA: Right atrium, AO: Aorta, PA: Pulmonary artery.

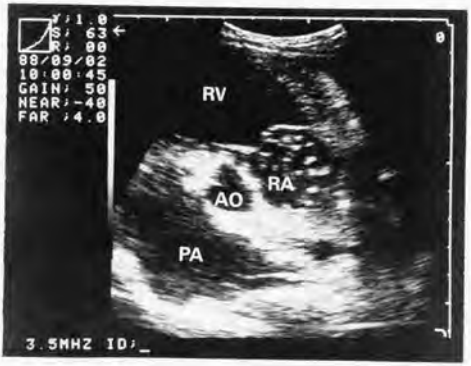

Fig. I-2. B-mode echocardiogram at the systolic phase in a spontaneous case. Heartworm located within the right atrium. RV: Right atrium, RA: Right atrium, AO: Aorta, PA: Pulmonary artery.

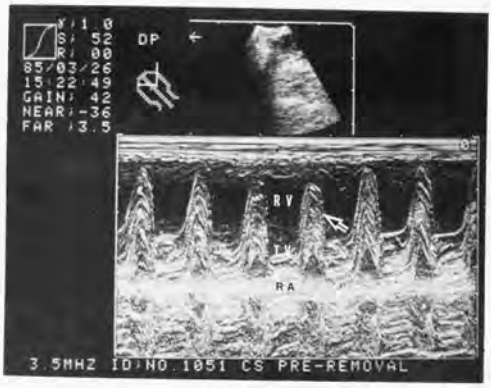

Fig.I-3. M-mode echocardiogram in a spontaneous case. Heartworm echoes move reciprocatedly between the right atrium and right ventricle. RV: Right ventricle, TV: Tricuspid valve, RA: Right atrium.

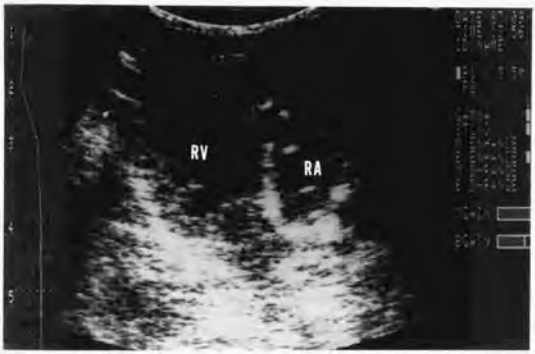

Fig. I-4. B-mode echocardiogram in a spontaneous case harboring a small number of heartworms. A few spotted echoes are observed at the tricuspid valve orifice and right atrium.

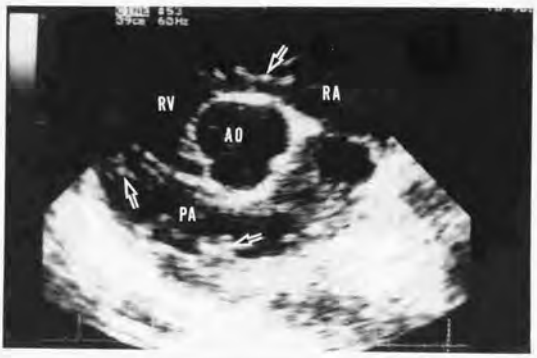

Fig. I-5. B-mode echocardiogram in a spontaneous case harboring heartworms also in the pulmonary arteries. Heartworm echoes (allow) are observed both at the tricuspid valve orifice and in the pulmonary arteries.

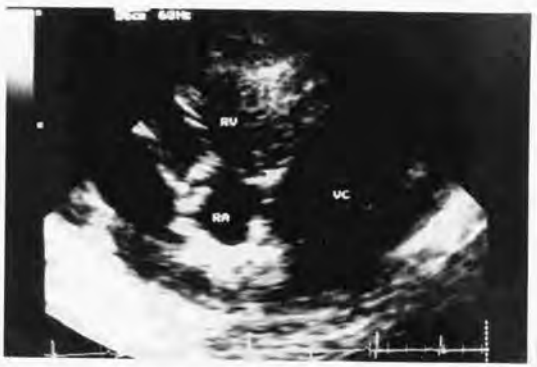

Fig. I-6. B-mode echocardiogram in a spontaneous case without the worms at the venae cavae. RV: Right ventricle, RA: Right atrium, VC: Venae cavae.

moved form the right atrium to the right ventricle at the diastolic phase, and returned to the right atrium at the systolic phase (Figs. I-1 to 3). A few spotted echoes were observed in the right atrium and at the tricuspid valve orifice in dogs harboring a small number of worms (Fig. I-4). In dogs with small number of heartworms, the heartworm echoes revolved in the right atrium, and a part of them were frequently projected into the right ventricle. The presence of heartworm echoes with reciprocating motion through the tricuspid valve orifice was the specific finding in this disease. In most but not all dogs, filarid worm echoes were observed also in the pulmonary arteries (Fig. I-5). Heartworm echoes could not be observed in the venae cavae of some dogs (Fig. I-6).

Table I-1 shows number of heartworms removed by surgical treatment by use of a flexible alligator forceps. Two to 72 worms were removed from the right atrium and tricuspid valve orifice. All worms were removed from the right atrium

Table I-1. Number of heartworms removed using a flexible alligator forceps

Dog No.	Body weight (kg)	No. of worms removed			No. of worms left in PA	Grand total	Grand total/ BW[c]
		RA and TVO[a]	PA[b]	Total			
1493	11.5	8 (6[d], 2[e])	18 (10, 8)	26	0 (0, 0)	26	2.26
1498	14.5	14 (12, 2)	1 (1, 0)	15	3 (0, 3)	18	1.24
1500	8.0	20 (14, 6)	0 (0, 0)	20	1 (1, 0)	21	2.63
1509	4.0	13 (8, 5)	3 (0, 3)	16	2 (0, 2)	18	4.50
1515	12.0	22 (21, 1)	2 (1, 1)	24	11 (1, 10)	35	2.92
1558	5.5	15 (5, 10)	1 (0, 1)	16	1 (0, 1)	17	3.09
1561	4.0	30 (28, 2)	28 (8, 20)	58	0 (0, 0)	58	14.50
1563	14.2	72 (70, 2)	44 (42, 2)	116	9 (0, 9)	125	8.80
1581	5.0	26 (18, 8)	31 (14, 17)	57	0 (0, 0)	57	11.40
1596	7.5	19 (19, 0)	7 (1, 6)	26	6 (1, 5)	32	4.27
1602	7.6	14 (9, 5)	5 (0, 5)	19	3 (0, 3)	22	2.89
1636	5.0	15 (15, 0)	10 (3, 7)	25	4 (1, 3)	29	5.80
1639	17.5	7 (5, 2)	0 (0, 0)	7	3 (0, 3)	10	0.57
1640	6.5	29 (10, 19)	0 (0, 0)	29	1 (0, 1)	30	4.62
1673	11.0	43 (33, 10)	0 (0, 0)	43	18 (10, 8)	61	5.55
1691	9.0	7 (3, 4)	1 (1, 0)	8	1 (1, 0)	9	1.00
1700	5.2	2 (0, 2)	0 (0, 0)	3	0 (0, 0)	3	0.58
1731	13.0	2 (2, 0)	0 (0, 0)	2	2 (0, 2)	4	0.31
1760	9.5	10 (10, 0)	11 (2, 9)	21	0 (0, 0)	21	2.21
Mean	9.0	19.3	8.6	27.4	3.4	31.4	3.64

a) Right atrium and tricuspid valve orifice. b) Pulmonary arteries. c) Body weight.
d) No. of female worms. e) No. of male worms.

and tricuspid valve orifice. From the pulmonary arteries, 0 to 44 worms were removed. The number of worms removed from the right atrium and tricuspid valve orifice was greater than from the pulmonary arteries in almost all dogs, but that from the pulmonary arteries was greater than from the right atrium in 3 dogs (Nos. 1493, 1581 and 1760). Most dogs harbored relatively

large numbers of heartworms, but several dogs (Nos. 1498, 1509, 1558, 1639, 1691, 1700 and 1731) harbored a small number of worms (less than 20 worms), indicating that the disease was not always followed by the harboring of a large number of worms.

Table I-2 shows location of adult heartworms in the cardiovascular system in 13 dogs with spontaneous CS examined by autopsy. The worms were consistently found in the venae cavae, right atrium, tricuspid valve orifice and right ventricle in all dogs. Worms were also noted in the pulmonary arteries and jugular or hepatic veins in some cases. More than half the worms were in the venae cavae and right ventricle. However, many were also found in the pulmonary arteries.

Table I-2. Location of heartworms in cardiovascular system of autopsied dogs

Dog No.	Number of heartworms			
	Total	Venae cavae~ right atrium	Right ventricle	Pulmonary artery
K- 2	86	54	5	32
K-27	160<	70[a)]	60<	30<
69	79	28	44	7
93	78	31	35	12
94	32	5	27	
143	54	20	34	
144	27	17	10	0
145	144	52	9	53
178	120<	40<	50<	30<
195	49	7	29	13
197	86	31	19	36
198	106	58	12	36
206	41	37	3	1

a) Approximately 50 worms were removed surgically before autopsy.

2) Right heart hemodynamics in spontaneous cases

On angiocardiography, the contrast medium which were infused into the right ventricle, regurgitated into the right atrium (Fig. I-7). The regurgitation disappeared following heartworm removal in almost all dogs (Fig. I-8). Selective arteriography of the pulmonary arteries revealed marked dilation, torture, abrupt pruning and obstruction of the arteries in almost all cases (Fig. I-9).

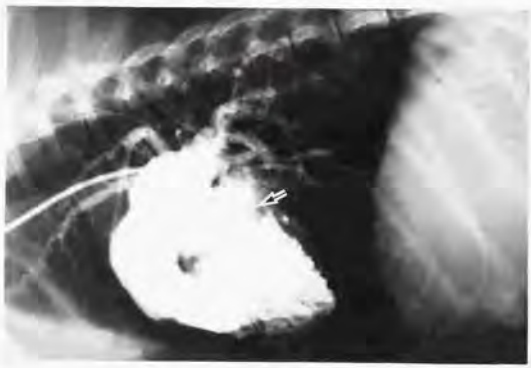

Fig. I-7. Left-lateral angiocardiogram in a spontaneous case before heartworm removal. The contrast medium regurgitated severely from the tricuspid valve orifice (allow).

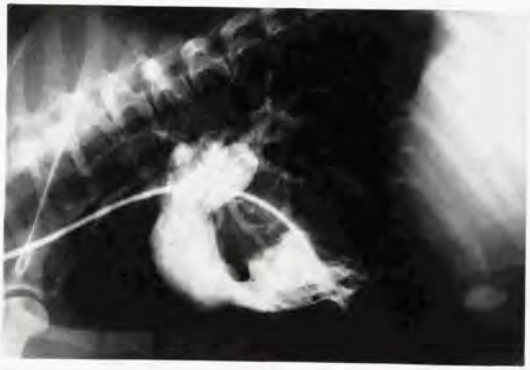

Fig. I-8. Left-lateral angiocardiogram in a spontaneous case after heartworm removal. Regurgitation of the contrast medium disappeared.

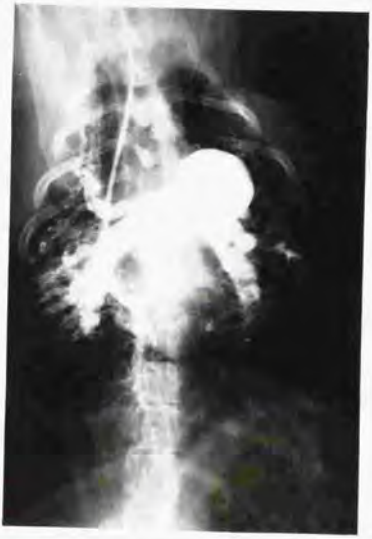

Fig. I-9. Dorso-ventral arteriogram in a spontaneous case. The pulmonary arteries were dilate, tortuate, abruptly pruned and obstructed.

Table I-3 shows central venous pressure (CVP) before and immediately after heartworm removal. In 19 dogs recovered after heartworm removal, the CVP ranged relatively higher level from 32 to 187 mmH_2O with a mean of 101.6±35.1 mmH_2O. The CVP fell immediately after heartworm removal, ranging from -10 mmH_2O to 130 mmH_2O with a mean of 61.0±42.9 mmH_2O. There was a significant difference between the CVP levels before and after heartworm removal ($p<0.01$). Systolic cardiac murmur and

Table I-3. Central venous pressure before and immediately after heartworm removal

Result	Dog No.	Central venous pressure (mmH_2O)			No. of worms removed
		Before removal	After removal	Discrepancy	
Recovery	268	100	84	16	23
	273	112	98	14	32
	275	90	51	39	50
	280	132	92	40	77
	281	121	97	24	22
	282	116	101	15	58
	283	32	-5	37	33
	284	46	17	29	63
	285	96	26	70	49
	286	106	54	52	78
	533	111	50	61	38
	540	56	-10	66	33
	542	112	87	25	20
	551	134	111	23	17
	560	63	3	60	50
	563	117	104	13	22
	571	187	130	57	48
	596	92	30	62	63
	597	107	39	68	58
	Mean	102	61	41	44
	SD[a]	35	43	21	19
Death	279[b]	216	200	16	22
	530[b]	254	175	79	64
	549[b]	66	61	5	47
	Mean	179	145	33	44
	SD	99	74	40	21

a) Standard deviation. b) Dead case.

jugular pulse disappeared in all dogs recovering after heartworm removal. In 3 dogs which died after heartworm removal, the CVP level before heartworm removal was extremely high in 2 dogs (Nos. 279 and 530). In these 2 dogs, CVP fell, but remained above 200 mmH_2O after heartworm removal. In another dog that died 15 hours after heartworm removal (No. 549), the CVP level before removal was 66 H_2O, and it fell only 5 mmH_2O immediately after removal. In this dog, 47 heartworms were removed, but systolic cardiac murmur and jugular pulse evidenced no recovery, and heartworm-coiling was noticed around the tricuspid valve chordae on autopsy.

Table I-4 shows pressures in the right heart system before and after heartworm removal. After heartworm removal, 8 dogs recovered (recovery group), and 10 dogs died or were euthanatized because of poor prognosis. Systolic and mean pulmonary arterial pressures ranged widely before heartworm removal, and those in the dead group were higher than in the recovery group. The systolic pulmonary arterial pressure remained higher level 1 day and 1 week after removal, but the mean pressure showed statistically significant fell 1 week after removal in the recovery group. There were no significant differences in pulmonary arterial pressures before and after heartworm removal in the dead group. Endodiastolic right ventricular pressure tended to decrease in both groups after heartworm removal. On right atrial pressure curve, elevation of v-wave was obvious in almost all dogs before heartworm removal (Fig. I-10).

Table I-4. Pressures in right heart system before and after heartworm removal

Item	Group	Before removal n	Mean	SD[a]	1 day after removal n	Mean	SD	1 week after removal n	Mean	SD
Systolic pulmonary arterial pressure (mmHg)	Recovery	8	61.7 (117.8-32.3)[b]	34.0	8	59.9 (107.8-28.6)	32.1	7	58.9 (116.6-30.3)	33.0
	Dead	10	81.8 (134.7-35.5)	34.9	10	80.6 (142.8-28.6)	34.6	7	76.6 (106.8-46.6)	21.8
Mean pulmonary arterial pressure (mmHg)	Recovery	8	38.7 (64.1-20.1)	15.7	8	36.8 (57.3-19.4)	15.2	7	34.9 (65.7-20.2)	16.2*
	Dead	10	48.7 (71.4-22.2)	17.2	10	48.7 (81.2-19.0)	17.2	7	46.7 (59.8-25.5)	12.8
Endodiastolic right ventricular pressure (mmHg)	Recovery	8	7.0 (22.2- -1.5)	8.5	8	7.2 (38.0- -3.7)	13.5	7	5.1 (20.3-0.7)	7.1
	Dead	10	17.3 (24.5-7.3)	6.0	10	16.9 (29.1-5.0)	8.9	7	12.3 (23.4- 1.2)	7.6
Right atrial pressure a-wave (mmHg)	Recovery	8	9.3 (32.9- 1.9)	10.7	8	5.5 (21.8- -1.2)	7.2	7	4.4 (14.0- 0.5)	4.8
	Dead	10	15.8 (23.8- 5.6)	6.1	10	14.7 (23.3- 2.3)	7.9	7	10.1 (17.1- 0.4)	6.3*
Right atrial pressure v-wave (mmHg)	Recovery	8	8.4 (27.6- 1.3)	10.3	8	4.3 (18.8- 0.1)	6.4	7	2.8 (11.2- -1.1)	4.2*
	Dead	10	21.4 (37.0-12.9)	6.9	10	18.3 (32.3- 6.4)	9.1	7	11.1 (16.6- 7.3)	3.7**

a) Standard deviation. b) Range. *, **) Probability of significant difference from the level before heartworm removal; each asterisk indicates $p<0.05$ and $p<0.01$, respectively.

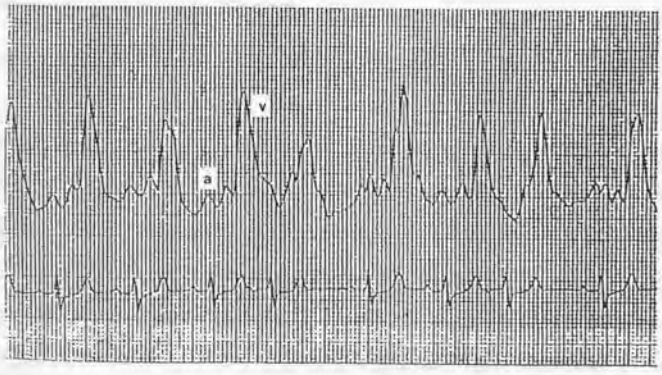

Fig. I-10. Pressure curve at the right atrium.
a: a-wave, v: v-wave.

Pressures of a- and v-wave in the right atrium ranged widely but were at relatively higher levels both in the recovery and dead groups. The right atrial pressures in the dead group were higher than that in the recovery group. Different from pulmonary arterial pressures, right atrial pressures fell after heartworm removal both in the recovery and dead groups. However, the pressures were still higher in the dead group than in the recovery group even 1 week after heartworm removal.

As shown in Table I-5, right cardiac output and other cardiac output indices indicated lower levels before heartworm removal. Cardiac output increased significantly after heartworm removal in the recovery group. In the dead group, however, cardiac outputs remained at lower levels 1 day after removal, but tended to increase 1 week after removal. Increase in right cardiac output tended to correspond roughly with the overall improvement of symptoms in dogs.

Table I-6 indicates heart rate and total pulmonary resistance. Heart rate did not change significantly after heartworm removal in the recovered and dead groups. Total pulmonary resistance ranged widely from 25210 to 4332 dynes·sec·cm^{-5}·kg in the recovery group, and from 36534 to 10047 dynes·sec·cm^{-5}·kg in the dead group. The resistance decreased after heartworm removal in the recovery group. The resistance did not change 1 day after removal, but decreased 1 week after removal in the dead group. The right ventricular stroke work index did not show the uniform changes either in

the recovery or dead group.

Table I-5. Right cardiac output before and after heartworm removal

Item	Group	Before removal n	Mean	SD[a]	1 day after removal n	Mean	SD	1 week after removal n	Mean	SD
Cardiac output (l/min)	Recovery	8	1.85 (2.58-1.21)[b]	0.50	8	2.11 (2.94-1.54)	0.54**	7	2.33 (3.34-1.77)	0.57*
	Dead	10	1.89 (3.54-1.00)	0.72	10	1.89 (3.29-1.06)	0.63	7	2.25 (2.90-1.51)	0.52
Cardiac index (ml/min/kg)	Recovery	8	271 (373-181)	77	8	316 (495-195)	106*	7	324 (453-221)	79**
	Dead	10	204 (299-117)	59	10	200 (273-151)	36	7	257 (349-155)	72
Stroke volume (ml/beat)	Recovery	8	12.6 (24.4-5.9)	6.0	8	14.7 (27.9-6.8)	6.7*	7	15.4 (27.5-7.6)	6.6
	Dead	10	12.8 (20.2-6.8)	4.4	10	13.7 (25.8-8.8)	4.9	5	16.1 (23.2-10.9)	4.3

a) Standard deviation. b) Range. *, **) Probability of significant difference; each asterisk indicates $p<0.05$ and $p<0.01$, respectively.

Table I-6. Heart rate, total pulmonary resistance and right ventricular stroke work index before and after heartworm removal

Item	Group	Before removal n	Mean	SD[a]	1 day after removal n	Mean	SD	1 week after removal n	Mean	SD
Heart rate (beat/min)	Recovery	8	160 (231-106)[b]	38	8	156 (228-105)	37	7	163 (234-122)	36
	Dead	10	149 (191-112)	24	10	138 (153-121)	11	7	142 (167-117)	20
Total pulmonary resistance (dyns·sec·cm^{-5}·kg)	Recovery	8	12215 (25210- 4332)	7316	8	9088 (20656- 4835)	5583*	7	9710 (23758- 3693)	6825**
	Dead	10	20308 (36534-10388)	9682	10	20342 (36581- 8604)	9622	7	15970 (23177- 6617)	6798*
Right ventricular stroke work index (g/beat/kg)	Recovery	8	0.85 (1.53-0.40)	0.40	8	1.00 (1.72-0.36)	0.48	7	0.93 (1.45-0.45)	0.37
	Dead	10	0.95 (2.24-0.31)	0.54	10	0.96 (1.73-0.34)	0.39	7	1.12 (2.01-0.77)	0.42

a) Standard deviation. b) Range. *, **) Probability of significant difference from the level before removal; each asterisk indicates $p<0.05$ and $p<0.01$, respectively.

3) Macroscopic findings in the right heart of spontaneous cases

Table I-7 shows principal macroscopic findings in dogs with spontaneous CS. There were no embolisms in the pulmonary arteries with dead heartworms in one case (No. 1581), but slight ones were found in 3 cases (Nos. 1509, 1561 and 1700). However, 15 other cases showed mild to severe pulmonary arterial embolisms with dead worms. Almost all embolisms were relatively fresh including heartworms that had recently died (Fig. I-11). Proliferation and thickness of the pulmonary arterial vessels (18/19 cases), as well as the thickness and hardening of the tricuspid valve leaflets (16/19 cases) were

Table I-7. Macroscopic findings in dogs with spontaneous caval syndrome

Dog No.	Time till autopsy (day)	Embolism with dead worms of PA[a)]	Proliferation and thickness of PA	Thickness and hardening of TV[b)]	Dead worm-coiling around TV chord
1493	7	++	++	+	−
1498	7	++	+	++	+
1500	7	+++	+++	+	++
1509	7	+	+++	++	−
1515	7	+++	+++	+++	−
1558	7	++	+	+++	+
1561	7	+	++	+	−
1563	14	++	++	++	−
1581	7	−	++	−	−
1596	7	+++	++	++	−
1602	14	+++	+	+	−
1636	7	++	+	−	−
1639	28	++	+	++	−
1640	10	+++	−	−	−
1673	1	+++	+++	+++	−
1691	3	+++	+	+	+
1700	6	+	++	+	−
1731	14	+++	+++	+++	−
1760	7	++	+++	+++	−

a) Pulmonary artery. b) Tricuspid valve.
c) Degree of injuries
−: No lesions, +: Slight lesion, ++: Mild lesion, +++: Severe lesion.

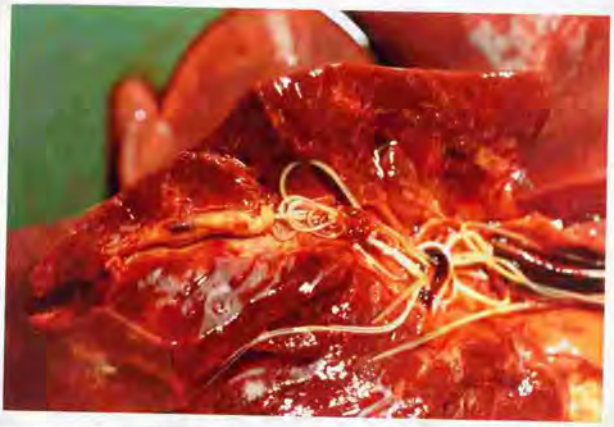

Fig. I-11. Pulmonary arterial embolism with dead heartworms.

were also observed. Dead heartworm-coiling around the tricuspid chord was found in 3 cases (Nos. 1498, 1500 and 1558). Dogs having a high pulmonary arterial pressure tended to have more severe and widespread embolisms of the pulmonary arteries with dead heartworms (Fig. I-12). However, there were no significant relationships among the pulmonary arterial pressure and other pulmonary lesions or number of heartworms harboring. Cardiac output did not correlated with the number of worms or any lesion in the pulmonary arteries.

4) Changes in cardiopulmonary function values after insertion of heartworm-like silicone tubes into the tricuspid valve orifice and right atrium

In order to investigate whether clinical signs and hemodynamic abnormalities were induced by interference with

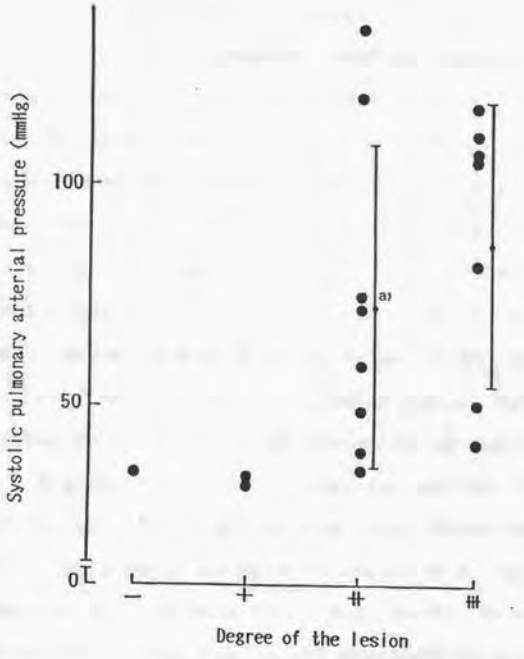

Fig. I-12. Relationship between the degree of pulmonary arterial embolisms with dead heartworms and systolic pulmonary arterial pressure

Degree of lesion; -: Negative, +: Slight, ++: Mild, and +++: Severe. a) Mean±SD.

tricuspid valve function, pseudo-heartworms made of silicone tubes were inserted into the tricuspid valve orifice and right atrium. In 3 dogs in which a small number of tubes (7 to 11 tubes) were inserted, tubes located mainly in the right atrium, and a small part of the tubes located at the tricuspid valve orifice (Fig. I-13). In 3 dogs in which a large number of tubes (29 to 37 tubes) were inserted, many tubes located at the orifice (Fig. I-14). The symptoms of so-called "caval syndrome" such as weakness, anorexia, paleness of the visible mucous membranes, systolic cardiac murmur, and jugular pulsation occurred following insertion of tubes in all or almost all cases (Table I-8). These symptoms were observed to the same degree in the small-number and large-number groups. Dyspnea was observed at a high prevalence, and ascites was observed in all dogs in which a large number of tubes were inserted, but not in dogs inserted a small number of tubes. However, urine hemoglobin was detected in the large-number group, but not in the small-number group. Details on urine hemoglobin detection will be presented in section III.

Regurgitation of a contrast medium from the right ventricle to the right atrium was observed (Fig. I-15). Systolic pulmonary arterial pressure did not show uniform changes after tube insertion (Fig. I-16). Pressure of a-wave of the right atrium (Fig. I-17) elevated slightly in all dogs. Elevation of a-wave was greater in the large-number group than in the small-number group. Besides, v-wave, which indicated regurgitation at the tricuspid valve orifice, elevated

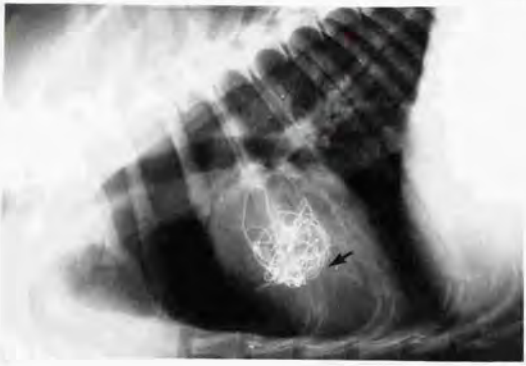

Fig. I-13. Location of heartworm-like silicone tubes in dogs inserted a small number of tubes. Arrow: Tricuspid valve.

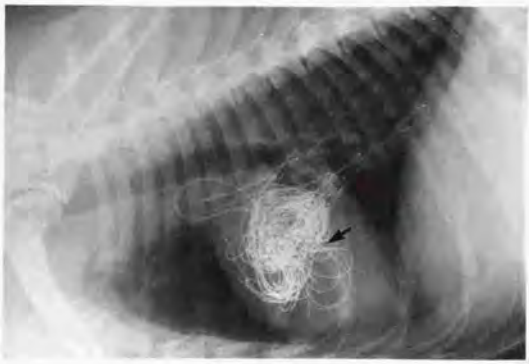

Fig. I-14. Location of heartworm-like silicone tubes in dogs inserted a large number of tubes. Arrow: Tricuspid valve.

Table I-8. Incidence of clinical signs after insertion of silicone tubes.

Sign	Small-number group (n=3)				Large-number group (n=3)			
	Before	1 day	3 days	7 days	Before	1 day	3 days	7 days
Weakness	0	2	3	3	0	3	3	3
Anorexia	0	2	3	3	0	3	3	3
Paleness of visible mucous membranes	0	3	3	3	0	3	3	3
Jugular pulsation	0	3	3	3	0	3	3	3
Cardiac murmur	0	3	3	3	0	3	3	3
Dyspnea	0	0	1	1	0	2	2	3
Ascites	0	0	0	0	0	0	1	3

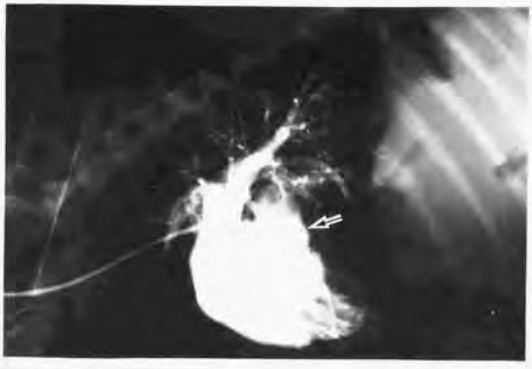

Fig. I-15. Regurgitation of contrast medium at the tricuspid valve orifice (arrow) in a case inserted heartworm-like silicone tubes.

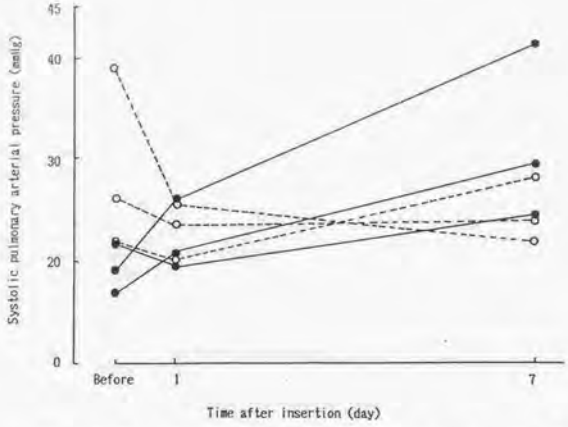

Fig. I-16. Changes in systolic pulmonary arterial pressure after insertion of heartworm-like silicone tubes
○--○: Case inserted a small number of silicone tubes.
●—●: Case inserted a large number of silicone tubes.

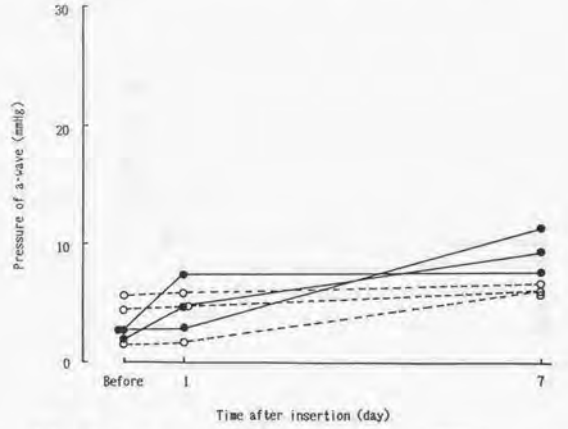

Fig. I-17. Changes in a-wave of right atrial pressure curve after insertion of heartworm-like silicone tubes
○--○: Case inserted a small number of silicone tubes.
●—●: Case inserted a large number of silicone tubes.

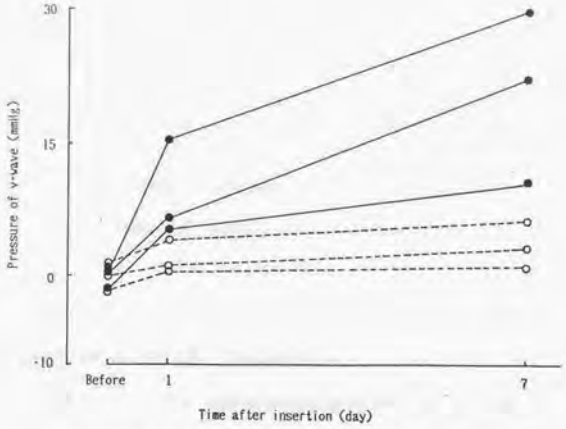

Fig. I-18. Changes in v-wave of right atrial pressure curve after insertion of heartworm-like silicone tubes
o--o: Case inserted a small number of silicone tubes.
●—●: Case inserted a large number of silicone tubes.

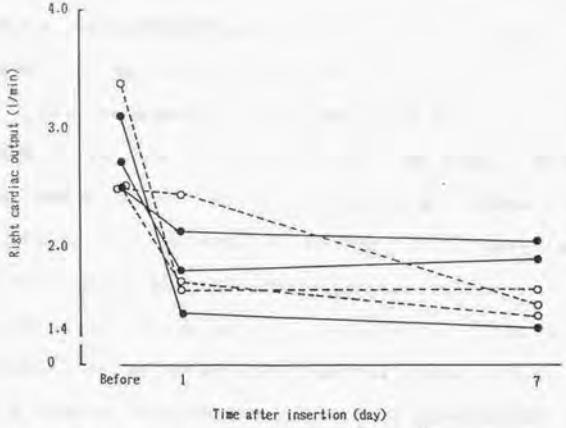

Fig. I-19. Changes in right cardiac output after insertion of heartworm-like silicone tubes.
o--o: Case inserted a small number of silicone tubes.
●—●: Case inserted a large number of silicone tubes.

strikingly till 1 week after tube insertion in the large-number group, but the elevation was mild in the small-number group (Fig. I-18). Right cardiac output decreased obviously in all dogs after tube insertion (Fig. I-19). Insertion of a large number of tubes greatly interfered with tricuspid valve function, and produced more severe symptoms of CS.

Discussion

In dogs with CS, heartworms located through the right heart system from the pulmonary arteries to the venae cavae. Since heartworms were observed in the venae cavae and right atrium on necropsy, the disease was termed "venae cavae syndrome" or "caval syndrome" [6, 30, 31, 33], and investigators attributed the cause of this disease to the circulatory disturbance owing to the embolization with live heartworms in the venae cavae. On the other hand, Buoro and Atwell [10] suggested that the habitat of heartworms associated with the onset of CS was the right atrium, from findings based upon insertion of a large number of rubber threads into the pulmonary artery and right atrium and removal of threads from the right atrium.

Certainly, heartworms located also in the right atrium and venae cavae in naturally acquired cases. Also, the dogs, in which heartworm-like silicone tubes positioned mainly in the right atrium, revealed symptoms of CS such as anorexia, and weakness. However, there were some spontaneous cases in which heartworms were rarely observed in the venae cavae on

echocardiography. Clinical signs such as systolic cardiac murmur and positive jugular pulsation indicated dysfunction of the tricuspid valve. Heartworms in the right atrium shall be caught at the tricuspid valve orifice and brings about the stenosis, because heartworms were invariably observed in the right ventricle through the tricuspid valve orifice at the diastolic phase on echocardiography. Regurgitation at the tricuspid valve orifice were also invariable. Regurgitation of the contrast medium infused into the right ventricle was noted in all dogs, along with a high CVP level dependent on the elevation of v-wave of right atrial pressure curve. Moreover, after surgical heartworm removal, symptoms indicating dysfunction of the tricuspid valve were alleviated, and right cardiac output increased in dogs recovered after treatment. Therefore, the habitat of heartworms related directly with the onset of CS must be the tricuspid valve orifice, but not the venae cavae or right atrium. Also, in an artificial model of the disease by Buoro et al. [10], rubber threads consistently located from the pulmonary arteries to the right atrium, and disturbed function of the tricuspid valve. The dysfunction of the tricuspid valve might cause symptoms to be reproduced.

The presence of heartworms at the tricuspid valve orifice brings about severe tricuspid valve dysfunction such as regurgitation and stenosis. Following tricuspid valve dysfunction, circulatory disturbance such as decrease in right cardiac output and increase in venous congestion resulted.

Besides, in the present study, pulmonary arterial embolisms with dead heartworms were found in almost all dogs with naturally acquired CS on autopsy. Embolization of the pulmonary arteries may cause circulatory disturbance through increase in pulmonary vascular resistance, and hypoxemia through ventilation disturbance in the lung. Pulmonary thromboembolism might contribute to development of the symptoms, and might be the underlying illness inducing heartworm migration from the pulmonary arteries. Moreover, when the heartworms are at the tricuspid valve orifice, cardiac output cannot increase because of tricuspid valve dysfunction, even if pulmonary vascular resistance decreases or pumping ability of the heart increases. Therefore, the presence of heartworms at the tricuspid valve orifice area may play the most important role in the development of the disease.

Because of severe circulatory disturbance, many organs are injured functionally and morphologically. Subsequent complications may be pulmonary, hepatic, and renal failures expressed as findings such as dyspnea, high plasma enzyme activities, abnormal plasma protein profiles and uremia [7, 25, 30]. The nature of clinical signs in dogs with CS shall be the low cardiac output, venous congestion and intravascular hemolysis. Intravascular hemolysis, another characteristic phenomenon of CS, will be discussed in SECTION III. After heartworm removal from the tricuspid valve orifice, the CVP fell immediately, right cardiac output increased, and clinical

signs were improved in dogs recovered after treatment. These findings also corroborated that circulatory disturbance play an important role in development of the symptoms. The number, position and conditions of heartworms at the tricuspid valve orifice may influence the valve function and right heart hemodynamics. Thus, the degree of symptoms varied widely in dogs with naturally acquired disease. Also, the period after onset may associate with the severity of signs. Many dogs with CS display the shock-like symptoms such as prostration, paleness of the visible mucous membranes, dyspnea, and low blood pressure. Atwell et al. [6] considered that the true nature of this disease was the "shock" involved. However, all dogs with this disease did not show such shock-like symptoms. The severity of clinical signs was roughly related with the decrease in right cardiac output in spontaneous and artificial cases. The shock-like symptoms may be complicated, but may be reversible in dogs recovered after heartworm removal and secondary from circulatory disturbance. In any cases, low cardiac output is the most important factor in development of signs of CS.

3. SECTION II. Mechanism of intravascular hemolysis

Introduction

Hemoglobinuria is one of the characteristic symptoms in CS indicating increase of intravascular hemolysis. Intravascular hemolysis has been found also in dogs with mild and chronic serious pulmonary dirofilariasis [25, 54]. Because the hemolysis in slight, obvious hemoglobinemia and hemoglobinuria cannot be observed in dogs with pulmonary heartworm disease. Besides, in almost all dogs with CS, because intravascular hemolysis is severe, symptoms following hemolysis such as hemoglobinemia, hemoglobinuria, anemia, and bilirubinuria are obvious. However, the mechanism of increase in intravascular hemolysis has not been clarified. As different from dogs with pulmonary heartworm disease, dogs with CS harbored heartworms in an unusual habitat; the tricuspid valve orifice between the right atrium and the right ventricle. It was considered that the worms at an unusual habitat might be associated with increase in intravascular hemolysis. In this section, in order to clarify the mechanism of intravascular hemolysis in dogs with CS, the author investigates relationships between the plasma hemoglobin concentration and location of heartworms in dogs with naturally acquired CS, and changes in plasma hemoglobin concentration after insertion of filaria-like silicone tubes at the tricuspid vavle orifice. Moreover, the hemolysis is discussed from findings of circulating erythrocyte shapes, erythrocyte fragilities, lipid contents in serum, lipoproteins and erythrocyte membranes and possible factors leading to

intravascular hemolysis including disseminated intravascular coagulation.

Materials and methods

Sixty-six dogs with naturally acquired CS (CS group) and 5 dogs developing the signs of CS after oral administration of milbemycin D at a dose of 1 mg/kg or 5 mg/kg body weight were used. Dogs revealed typical symptoms of CS such as cardiac murmur, jugular pulsation, prostration, labored respiration and/or hemoglobinuria. As controls, 93 healthy and heartworm-free dogs (heartworm-free group) and 6 dogs with subclinical pulmonary heartworm disease (subclinical group), 18 dogs with mild stage of pulmonary heartworm disease (mild group), and 28 dogs with signs of right heart failure (serious group) were used. For experiment of artificial heartworm insertion, 2 heartworm-free and 8 infected dogs were used. Seven to 12 heartworm-like silicone tubes were inserted into the right atrium and tricuspid valve orifice of 5 dogs (small-number group), and 25 to 37 tubes in another 5 dogs (large-number group).

Heartworm removal with venotomy was performed with a straight or flexible alligator forceps [17, 27]. Insertion of heartworm-like silicone tubes was performed by the same method described in section I.

Plasma hemoglobin concentration was determined by benzidine reaction [16]. Detection of hemoglobin and bilirubin in urine was performed with a regent strip (Multistix-III,

Miles-Sankyo Co. Ltd., Tokyo). Blood smear was prepared with May-Giemsa stain. Shapes of peripheral erythrocytes were observed also on the wet preparation. Osmotic fragility of erythrocytes were determined by use of the method of Parpart [35], and mechanical fragility by the method of Shen et al. [49]. Conventional liver function tests, including serum protein fractination, serum enzyme activities, serum bilirubin, urea nitrogen and glucose concentrations, were performed by cellulose acetate electrophoresis and with a RaBA-super system (Chugai Pharmaceutical Co. Ltd., Tokyo). Serum haptoglobin concentration (hemoglobin binding capacity) was also determined by celluose acetate electrophoresis [60]. Serum bile acid level was measured by the enzyme-fluorometric method (Strognost-3alpha kit, Nyegaard & Co., A/S, Oslo, Norway). Serum lecithin cholesterol acyltransferase (LCAT) activity was determined according to the method of Stokke and Norum [53] using ^{3}H-cholesterol. Lipids in sera, lipoproteins and erythrocyte membranes were analyzed by a thin-layer chromatography-flame ionization detector (TLC-FID) system (Iatroscan TH-10 TLC-FID analyser, Iatron Lab. Inc., Tokyo). Cholesterol and phospholipid contents were also determined by the chemical methods; O-phthalaldehyde method for cholesterol (Cholesterol B-test Wako, Wako Pure Chemical Industries Ltd., Osaka) and potassium permanganate wet combustion method (Phospho-lipid test Wako). Serum lipoproteins were fractionated by cellulose acetate electrophoresis, and serum total lipid concentration was determined by TLC-FID method.

Each lipoprotein fraction was quantitated by calculation from the percent composition of each lipoprotein and serum total lipid concentration according to the method of Ito et al. [29]. For analysis of lipoprotein lipid contents by use of TLC-FID method, lipoproteins were separated also by use of ultracentrifugation with reference to the method of Harvel et al. [23]. Serum osmolality was measured with a vapor pressure osmometer (Model 5100-B, Wescor Inc., Logan, Utah, U.S.A.). Blood ATP level was determined by the Beutler method [8].

Platelets were counted by the Brecher-Cronkite method [9]. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using commercial reagents for human blood coagulation tests (Simplastin, Warner-Lambert Co., Morris Plaines, New Jersey, or Actin, Dade Diagnostics Inc., Delaware Parkway, Miami) with a Micro-coagulometer (Greiner Electronics Co., Switzerland). Fibrinogen concentration was measured by thrombin time assay (Fibrinogen Determination Reagents, Dade Diagnostics), and factor VIII by APTT assay using a coagulation factor deficiency substrate plasma (Dade Diagnostics). Fibrinogen-fibrin degradation products (FDP) were determined by latex agglutination test (FDPL Test, Teikoku Hormone Manufacturing Co. Ltd., Tokyo). A histological examination was conducted on the liver, kidneys, lungs, heart, spleen and alimentary canals. These organs were fixed in 10 % formalin solution as soon as possible after euthanasia or death, embedded in paraffin, cut into 5 micrometer thickness, and stained with hematoxylin and eosin,

or phosphotangustic acid and hematoxylin for microscopic examination.

Results

1) Plasma hemoglobin concentration

Table II-1 shows plasma hemoglobin levels in the heartworm-free, mild, serious and CS groups. In the heartworm-free and mild groups, plasma hemoglobin concentration ranged lower levels with a mean of 4.2±2.5 mg/dl and 5.5±5.3 mg/dl, respectively. In the serious group, the concentration ranged from the normal to slightly higher levels. In the CS group, plasma hemoglobin concentration ranged from 11.0 mg/dl to 1020 mg/dl with a mean of 232±216 mg/dl.

Table II-1. Plasma hemoglobin levels

Item	Heartworm-free			Mild			Serious			Hemoglobinuria		
	n	Mean	SD[a]	n	Mean	SD	n	Mean	SD	n	Mean	SD
Plasma hemoglobin (mg/dl)	33	3.7 (0.7 - 8.2)[b]	2.4	18	5.5 (1.0 - 12.9)	5.3**	16	12.0 (3.6 - 19.0)	7.4**	64	232 (11.0 - 1020)	216

a) Standard deviation. b) Range. *, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

2) Relationship between the presence of heartworms at tricuspid valve orifice and plasma hemoglobin concentration

After surgical treatment, heartworm echoes disappeared from the tricuspid valve orifice and right atrium (Figs. II-1 and II-2). Table II-2 shows plasma hemoglobin concentrations

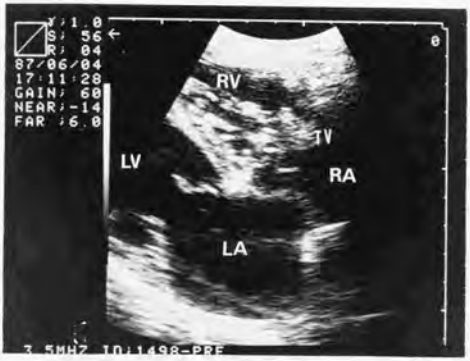

Fig. II-1. B-mode echocardiogram before heartworm removal in a spontaneous case. Heartworms locate at the tricuspid valve orifice between the right atrium and right ventricle. TV: Tricuspid valve orifice, RA: Right atrium, RV: Right ventricle, PV: Pulmonary valve, PA: Pulmonary artery.

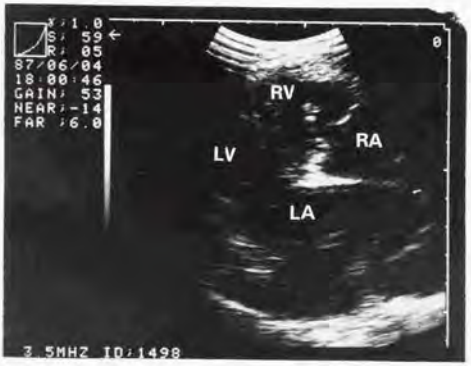

Fig. II-2. B-mode echocardiogram after heartworm removal in a spontaneous case. Heartworms disappeare from the tricuspid valve orifice.

Table II-2. Changes in plasma hemoglobin concentration with time after heartworm removal in dogs with spontaneous CS

Dog No.	Plasma hemoglobin concentration (mg/dl)						
	Before	30 min[a]	1 hr	2 hr	3 hr	6 hr	12 hr
960	113.5	104.2	101.8	100.8	101.0	67.6	19.4
963	312.3	214.7	268.1	159.6	94.1	19.1	9.9
989	239.0	219.6	198.1	223.9	147.5	69.6	8.1
990	250.5	241.2	209.5	170.0	131.2	40.8	5.7
996	55.0	44.0	43.8	43.4	23.7	23.1	29.1
1016	140.5	55.4	36.1	54.1	NM[b]	10.6	14.3
1050	147.9	129.2	87.1	70.3	26.6	2.2	0
1051	373.4	304.4	275.7	272.0	244.4	140.8	8.1
1069	142.4	109.5	105.8	84.1	57.3	8.5	9.2
1096	238.0	238.6	196.3	150.8	101.1	57.3	7.6
991[c]	677.9	613.2	604.6	NM	NM	NM	NM
997[c]	968.8	925.7	904.2	NM	NM	NM	NM
Mean	304.9	266.6	252.4	132.9	103.0	44.0	11.1
SD[d]	265.2	257.0	255.8	75.7	68.1	42.0	8.1

a) Time after heartworm removal. b) Not measured. c) Dead case.
d) Standard deviation.

determined after heartworm removal. The concentrations began to decrease at 30 minutes after heartworm removal. The concentrations decreased gradually, and reached almost the normal level at 20 hr after heartworm removal.

In dogs developing CS after oral administration of milbemycin D, heartworm echoes the same as spontaneous cases were observed at the tricuspid valve orifice (Fig. II-3). Plasma hemoglobin concentrations ranged almost at normal levels before drug administration at which heartworms were

present in the pulmonary arteries. After recognition of heartworms at the tricuspid valve orifice, plasma hemoglobin concentrations elevated to different levels in all cases (Fig. II-4).

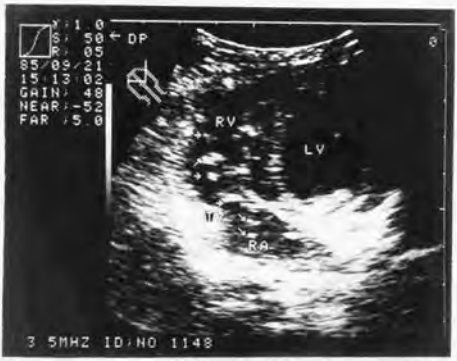

Fig. II-3. B-mode echocardiogram at the time of onset in a case induced heartworm migration by administration of milbemycin D. Heartworm echoes locate from the right ventricle to the right atrium. RV: Right ventrice, LV: Left ventricle, TV: Tricuspid valve, MV: Mitral valve, RA: Right atrium, LA: Left atrium, VC: Venae cavae.

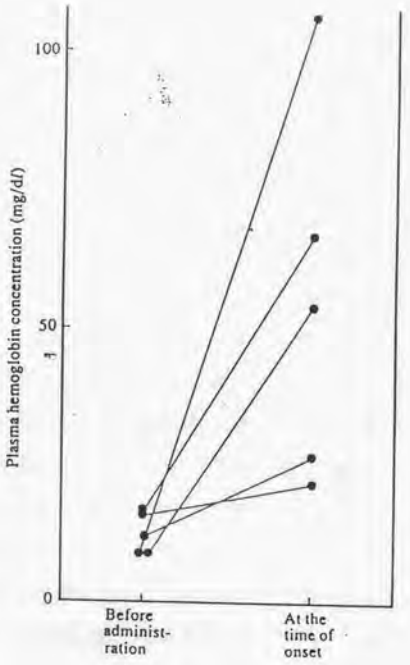

Fig. II-4. Plasma hemoglobin concentrations before administration of milbemycin D and at the time of onset

In order to induce intravascular hemolysis, heartworm-like silicone tubes were inserted into the right atrium and tricuspid valve orifice of dogs. Figures II-5 and II-6 show schematic locations of tubes duplicated from radiographs. In dogs of the small-number group in which a relatively small number of tubes (7 to 12 tubes) were inserted, tubes broke into the tricuspid valve orifice. A few tubes flow into the pulmonary arteries even 7 days after insertion in these dogs.

In dogs of the large-number group inserted a large number of tubes (25 to 37 tubes), many tubes broke into the tricuspid valve orifice at 1 day after insertion. Tubes flowed into the pulmonary arteries gradually, but a considerable number remained at the tricuspid vale orifice 7 days after insertion.

As shown in Fig. II-7, plasma hemoglobin concentration elevated markedly 1 day after insertion in the large-number group. Plasma hemoglobin concentration decreased, but remained at a relatively higher level (above 70 mg/dl) 7 days after insertion. Number of tubes at the tricuspid valve orifice increased at 7 days after insertion in one dog (No. 1693), and plasma hemoglobin concentration increased simultaneously. Plasma hemoglobin concentrations did not increase in the small-number group except in one dog (No. 1661), in which plasma hemoglobin concentration was 57 mg/dl 1 day after insertion of 10 tubes. As shown in Table II-4, urine hemoglobin was detected in all dogs of the large-number group and one dog of the small-number group (No. 1661). A dog (No. 1705) inserted 37 silicone tubes excreted obviously visible hemoglobinuria 1 day after insertion.

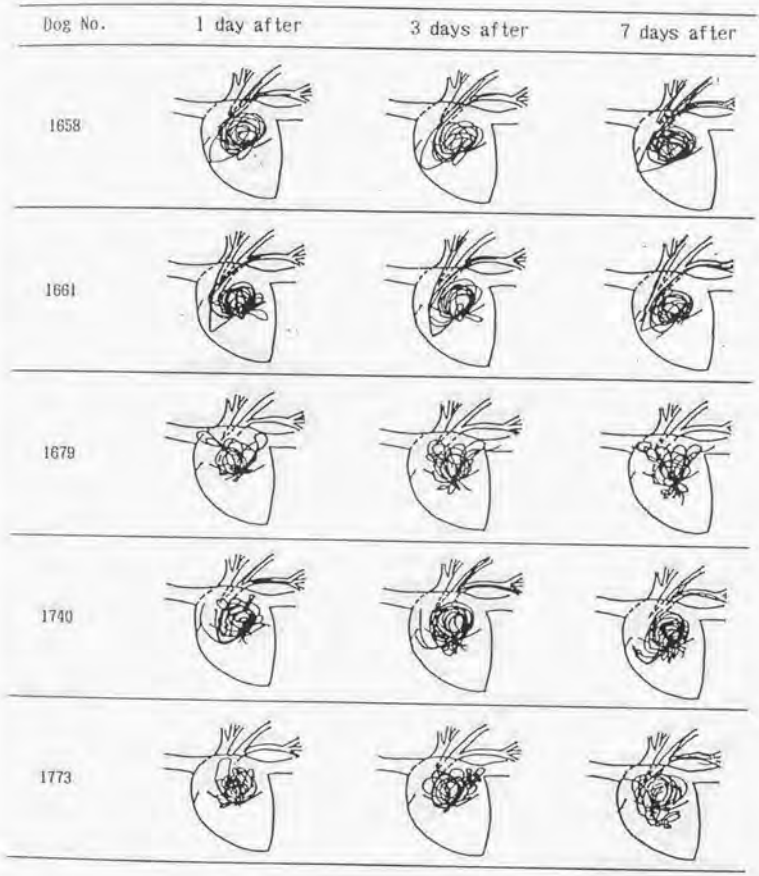

Fig. II-5. Schematic location of silicone tubes in dogs of the small-number group

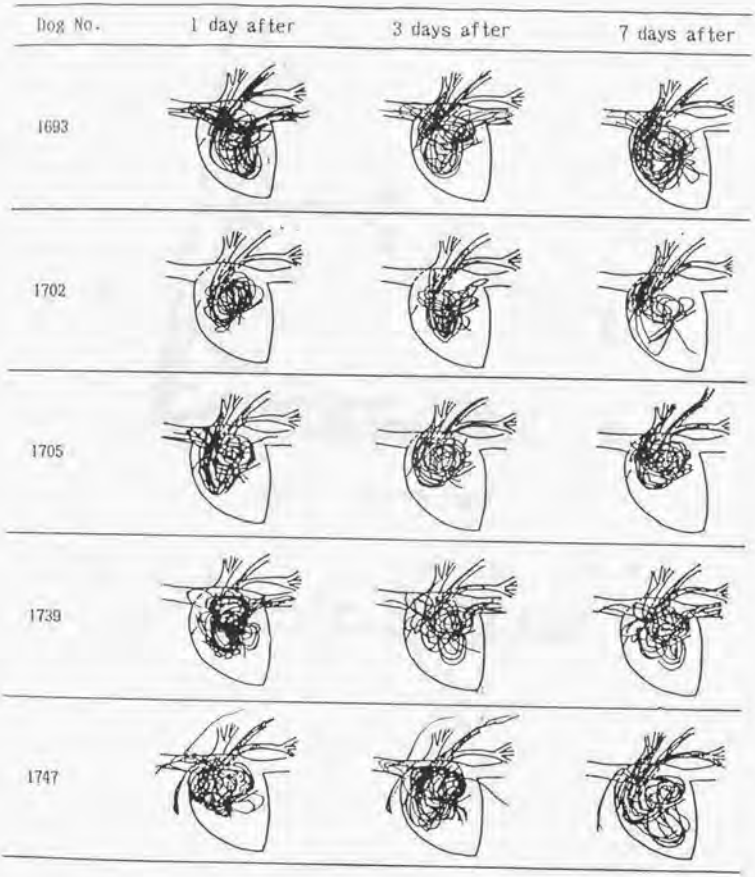

Fig. II-6. Schematic location of silicone tubes in dogs of the large-number group

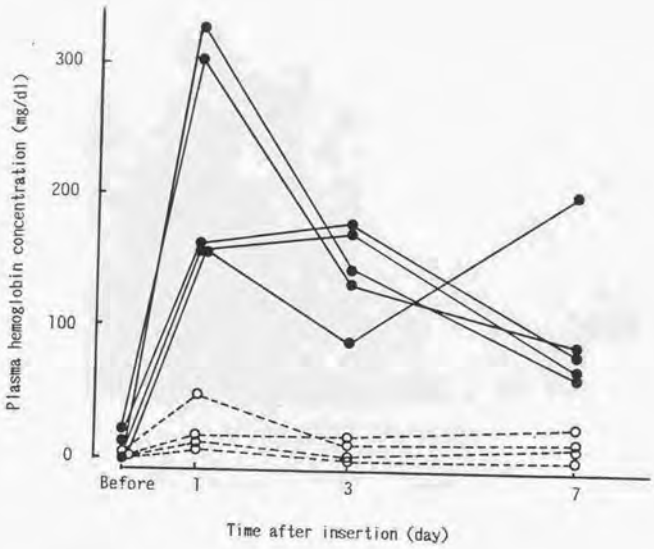

Fig. II-7. Plasma hemoglobin cancentration before and after insertion of silicone tubes
○--○: Case inserted a small number of silicone tubes.
●—●: Case inserted a large number of silicone tubes.

3) Shape of peripheral erythrocytes and erythrocyte fragilities

Various abnormal erythrocytes were observed in peripheral blood. Acanthocytes (spur cells, Fig. II-8), codocytes (target

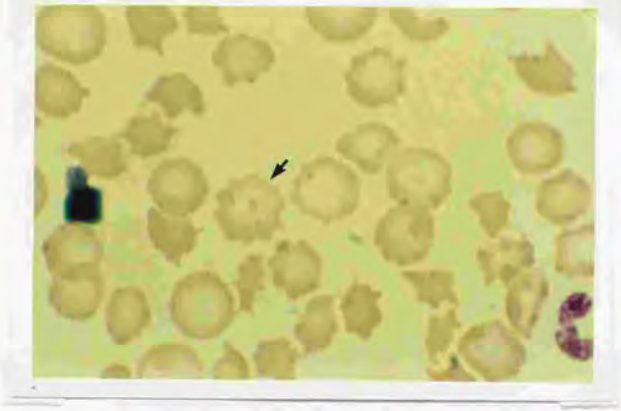

Fig. II-8. Acanthocytes observed on blood film (allow).

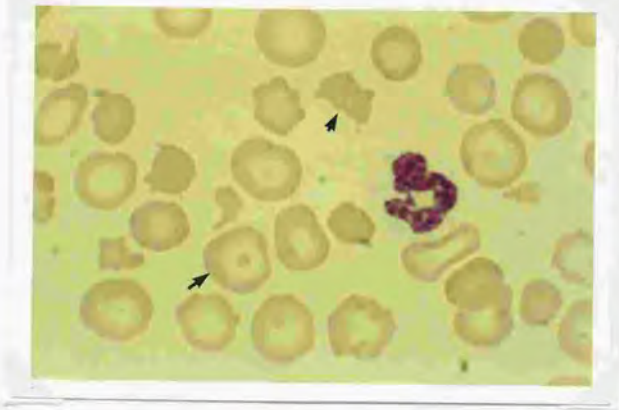

Fig. II-9. Codocytes (↗) and scizocytes (↑) observed on a blood film.

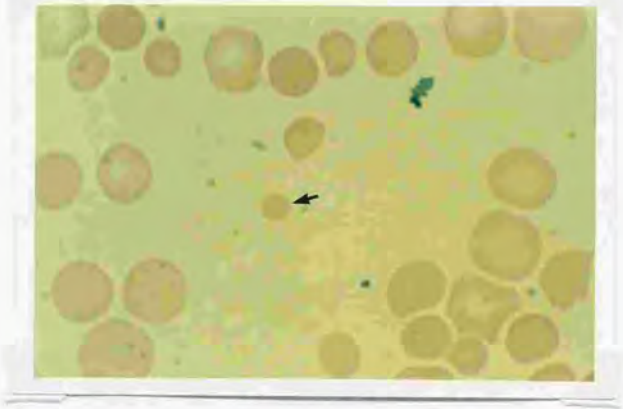

Fig. II-10. Microspherocyte observed on blood film (allow).

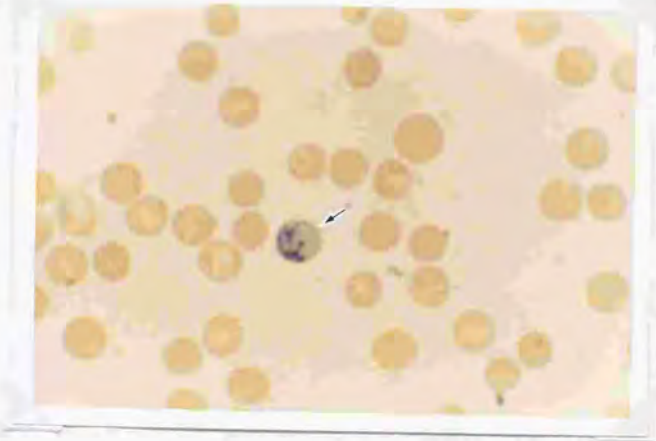

Fig. II-11. Reticulocyte observed on blood film (allow).

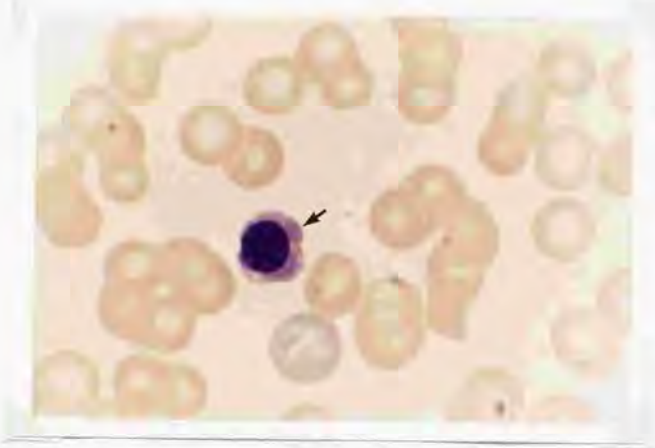

Fig. II-12. Erythroblast observed on blood film (allow).

cells) and scizocytes (schistocytes or fragmented cells, Fig. II-9) were observed at a relatively high incidence. Microspherocytes (Fig. II-10) were also found. Almost all cases revealed a considerable number of the reticulocytes (Fig. II-11) and erythroblasts (Fig. II-12).

Fig. II-13 shows osmotic fragility curves of circulating erythrocytes measured in each 6 dogs of the serious and CS groups. Mean fragility curve in the CS group indicated that the medium corpuscular fragility (50 % hemolysis) was normal, but 1 % hemolysis and 99 % hemolysis higher than in the normal group. Mean curve in the serious group was similar to that in the CS group. Table II-3 shows erythrocyte mechanical fragility in the normal, serious and CS groups. The fragility was significantly higher in the serious

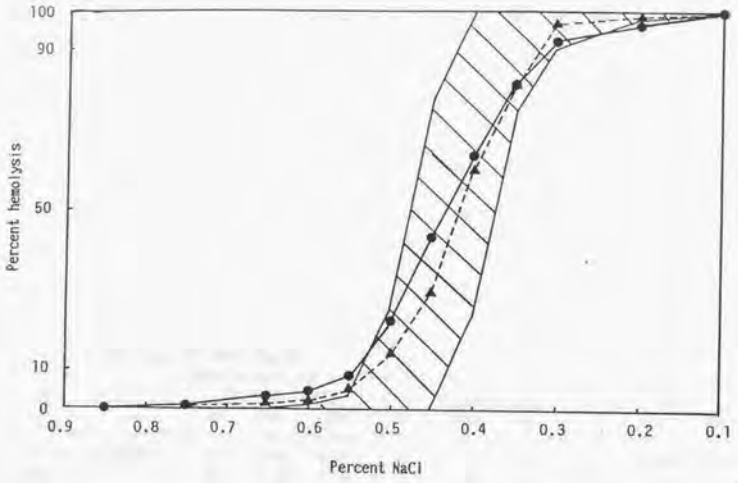

Fig. II-13. Erythrocyte osmotic fragility curves
●—●: CS group (Mean in 6 cases).
▲--▲: Serious group (Mean in 6 cases).
▨: Normal range (Mean±2SD in 12 heartworm-free dogs).

Table II-3. Erythrocyte mechanical fragility

Item	Heartworm-free			Serious			CS			p[b]
	n	Mean	SD[a]	n	Mean	SD	n	Mean	SD	
Erythrocyte mechanical fragility (% hemolysis)	17	2.6 (0.8 - 3.9)[d]	0.8	11	5.4 (3.5 - 6.7)	1.8**	26	5.2 (3.8 - 8.7)	1.5**	NS[c]

a) Standard deviation. b) Probability of significant difference between the serious and CS groups.
c) Not significant. d) Range. *, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

and CS groups than in the normal group. There were no significant differences between fragilities in the serious and CS groups.

4) Liver function test results

Table II-4 shows results of liver function tests. Compared with the heartworm-free group, serum albumin

Table II-4. Liver fiunction test results

Item	Heartworm-free			Serious			CS		
	n	Mean	SD[a)]	n	Mean	SD	n	Mean	SD
Total protein (g/dl)	93	6.64	0.44	59	6.51	1.44	65	7.09	0.96
Albumin (g/dl)	93	3.49	0.23	55	2.24	0.81	59	2.40	0.45
A/G ratio	92	1.08	0.23	55	0.55	0.24	59	0.53	0.17
Serum haptoglobin (mg/dl)	13	114.8	31.5	17	33.7	59.8	24	46.5	29.3
GOT (IU/L)	83	21.2	7.1	47	51.0	31.6	63	313	313
GPT (IU/L)	81	19.8	6.5	59	71.2	82.1	59	363	721
LDH (IU/L)	78	92.3	58.3	21	346.6	286.4	61	1104	657
ALP (IU/L)	64	72.7	28.6	14	262.6	362.6	60	531	614
Bilirubin (mg/dl)	38	0.30	0.13	6	0.25	0.04	64	2.34	3.12
Bile acid (nM)	94	5.3	3.9	14	38.0	40.8	11	29.4	23.4
BUN (mg/dl)	75	14.0	3.4	41	20.4	10.9	66	52.9	37.1
Glucose (mg/dl)	42	85.3	9.9	31	99.5	22.1	65	112.4	32.2

a) Standard deviation.

concentration and A/G ratio were lower in the serious and CS groups. Serum haptoglobin levels in the serious and CS groups were lower than in the heartworm-free group. However, serum haptoglobin did not disappear in spite of high plasma hemoglobin concentration in almost all dogs of the CS group.

Serum GOT, GPT, LDH and ALP activities ranged from normal to slightly higher levels in the serious group, but distributed at extremely higher levels in the CS group. Serum bile acid level was higher, but serum bilirubin, BUN and serum glucose levels were almost within the normal ranges in the serious group. Serum bilirubin, BUN and serum glucose levels were considerably high in the CS group. These data indicated severe and relatively acute liver dysfunction in dogs of the CS group.

Table II-5 shows serum LCAT activities in the heartworm-free, subclinical, mild and CS groups. Serum LCAT activity was 19.1±6.4 mcg/ml/hr in the normal group. The LCAT level was approximately 40 % and 50 % lower in the subclinical and mild groups, respectively, than in the heartworm-free group. The LCAT activities in the CS group ranges from 1.2 mcg/ml/hr to 12.5 mcg/ml/hr with a mean of 7.5±3.0 mcg/ml/hr, and was significantly lower than in the heartworm-free group.

Table II-5. Serum LCAT activities

Item	Heartworm-free			Subclinical			Mild			CS		
	n	Mean	SD[a)]	n	Mean	SD	n	Mean	SD	n	Mean	SD
Serum LCAT activity (μg/ml/hr)	28	19.1 (10.0-31.2)[b)]	6.4	6	12.3 (7.2-18.7)	4.3	8	8.8 (5.4-11.6)	2.2**	20	7.5 (1.2-12.5)	3.0**

a) Standard deviation. b) Range. *, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

5) Serum lipoprotein levels

Serum lipoprotein levels are shown in Table II-6. Alpha-lipoprotein (high density lipoprotein, HDL) level was lower, but pre-beta-lipoprotein (very low density lipoprotein, VLDL) and beta-lipoprotein (low density lipoprotein, LDL) levels were significantly higher in the CS group than in the heartworm-free group both in the percent composition and concentration.

Table II-6. Serum lipoprotein concentrations

Item	Heartworm-free			CS			p<[b]
	n	Mean	SD[a]	n	Mean	SD	
α-lipoprotein (%)	13	86.4	5.8	6	43.0	10.4	0.01
(mg/dl)		1221	127		567	126	0.01
Pre-β-lipoprotein (%)	13	8.9	4.7	6	32.9	11.8	0.01
(mg/dl)		81	46		302	166	0.01
β-lipoprotein (%)	13	4.7	2.6	6	24.2	7.8	0.05
(mg/dl)		54	30		269	95	0.01

a) Standard deviation. b) Probability of significant difference between the serious and CS groups. *, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

6) Lipids in serum, lipoproteins and erythrocyte membranes

Table II-7 shows serum lipid levels determined by TLC-FID method in dogs of the heartworm-free, serious and CS groups. Compared with the heartworm-free group, serum cholesterol ester level was lower, and free cholesterol level

Table II-7. Serum lipid levels

Item	Heartworm-free			Serious			Hemoglobinuria			p[b]
	n	Mean	SD[a]	n	Mean	SD	n	Mean	SD	
Cholesterol ester (mg/dl)	18	241	64	12	229	103	17	155	85**	0.01
Free cholesterol (mg/dl)	18	53	9	12	83	47	17	78	28**	NS[b]
Cholesterol ester ratio (%)	18	81.3	3.3	12	73.5	6.0**	17	65.8	11.1**	0.05
Triglyceride (mg/dl)	17	25	14	12	71	61*	17	37	21	NS
Phosphatidyl choline (mg/dl)	18	348	83	12	287	110	17	183	120**	0.01
Sphingomyelin (mg/dl)	18	29	6	12	36	11**	17	44	18**	0.01
Lysophosphatidyl choline (mg/dl)	18	25	7	12	18	6**	17	17	9**	NS
Total phospholipid (mg/dl)	18	403	92	12	355	143	17	244	128**	0.01
Total lipid (mg/dl)	17	719	160	12	739	326	17	513	234**	NS

a) Standard deviation. b) Probability of significant difference between the serious and CS groups. *, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

was higher, while cholesterol ester ratio was lower in the CS group. Cholesterol ester ratio in the CS group was higher than in the serious group. Phosphatidyl choline, lysophosphatidyl choline and total phospholipid concentrations were higher, but sphingomyelin level was lower in the CS group.

As shown in Fig. II-14, a negative correlation with relatively high significance ($p<0.01$) was found between the serum LCAT activity and serum free cholesterol concentration ($r=-0.75$). A positive and statistically significant ($p<0.01$) correlation was noticed between the serum LCAT activity and cholesterol ester ratio ($r=0.48$, Fig. II-15).

Lipid contents in each lipoprotein fraction separated by ultracentrifugation (Table II-8) indicated a decrease in cholesterol ester ration and lysophosphatidyl choline levels in HDL. All lipid levels in LDL of the CS group were higher than in the normal group. There were no significant

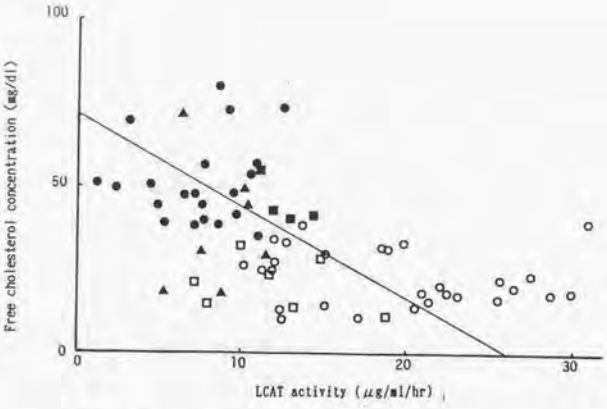

Fig. II-14. Correlation between the lecithin cholesterol acyltransferase activity and serum free cholesterol concentration
○: Heartworm-free case, □: Subclinical case,
▲: Mild case, ●: CS case.

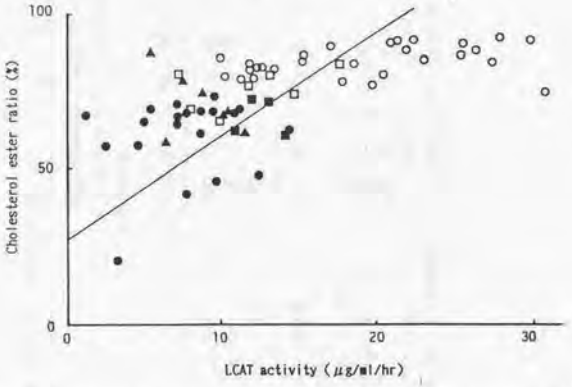

Fig. II-15. Correlation between the lecithin cholesterol acyltransferase activity and serum cholesterol ester ratio
○: Heartworm-free case, □: Subclinical case,
▲: Mild case, ●: CS case.

Table II-8. Lipid contents in lipoproteins

Item	High density lipoprotein							Low density lipoprotein							Very low density lipoprotein						
	Heartworm-free			CS				Heartworm-free			CS				Heartworm-free			CS			
	n	Mean	SD[a]	n	Mean	SD	p<[b]	n	Mean	SD	n	Mean	SD	p<	n	Mean	SD	n	Mean	SD	p<
Cholesterol ester (mg/dl)	7	246	31	4	150	121	NS	7	13	7	4	60	23	0.05	7	15	7	4	21	27	NS
Free cholesterol (mg/dl)	7	40	4	4	41	28	NS	7	7	2	4	32	5	0.01	7	9	3	4	17	13	NS
Cholesterol ester ratio (%)	7	86	1	4	78	3	0.05	7	66	7	4	64	8	NS	7	61	9	4	45	14	NS
Triglyceride (mg/dl)	7	2	1	4	3	1	NS	7	3	1	4	21	6	0.01	7	18	2	4	29	11	NS
Phosphatidyl choline (mg/dl)	7	342	29	4	225	103	NS	7	25	13	4	84	34	0.05	7	40	19	4	40	35	NS
Sphingomyelin (mg/dl)	7	23	3	4	24	21	NS	7	3	3	4	14	7	0.05	7	5	1	4	7	6	NS
Lysophosphatidyl choline (mg/dl)	7	28	5	4	13	5	0.01	7	0	0	4	5	3	0.01	7	3	2	4	1	2	NS
Total phospholipid (mg/dl)	7	393	33	4	263	128	NS	7	28	15	4	102	35	0.01	7	47	21	4	48	42	NS
Total lipid (mg/dl)	7	681	58	4	457	275	NS	7	51	23	4	215	56	0.01	7	83	28	4	115	90	NS

a) Standard deviation. b) Probability of significant difference between the heartworm-free and CS groups.

differences in lipid contents of VLDL.

Table II-9 shows lipid contents in erythrocyte membranes. Free cholesterol content increased in the serious and CS groups, but a significant difference was not found between the serious and CS groups. Phospholipid level was higher only in the serious group. Cholesterol/phospholipid ratio tended to be higher in the CS group than in the heartworm-free group.

Table II-9. Lipid contents in erythrocyte membranes

Item	Heartworm-free			Serious			CS			p[b]
	n	Mean	SD[a]	n	Mean	SD	n	Mean	SD	
Free cholesterol (mg/gHb)	18	5.93	0.53	12	8.51	1.80**	18	7.48	1.35**	NS
Phosphatidyl ethanolamine (mg/gHb)	18	2.76	0.84	12	4.15	2.77	18	2.64	1.35	NS
Phosphatidyl serine (mg/gHb)	18	1.42	0.51	12	2.16	0.78**	18	1.55	0.77	0.01
Phosphatidyl choline (mg/gHb)	18	7.39	1.51	12	9.15	3.22	18	7.56	1.47	NS
Sphingomyelin (mg/gHb)	18	0.63	0.29	12	1.29	1.08	18	10.9	0.48**	NS
Total phospholipid (mg/gHb)	18	12.14	2.27	12	16.75	7.35	18	12.84	2.70	NS
Total lipid (mg/gHb)	18	18.09	2.66	12	25.26	9.01*	18	20.32	3.37*	NS
Cholesterol/phospholipid ratio	18	0.47	0.08	12	0.55	0.12	18	0.60	0.16**	NS

a) Standard deviation. b) Probability of significant difference between the serious and CS groups.
*, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

Table II-10 shows relationships between the lipid contents in whole serum and each lipoprotein fraction. Cholesterol ester concentration in whole serum was significantly correlated with cholesterol ester content in HDL, and free cholesterol concentration in serum did with free cholesterol content in LDL. Triglyceride concentration correlated with triglyceride contents in all lipoprotein fractions. Correlation coefficients were relatively high between the phospholipid contents in whole serum and HDL.

Table II-10. Correlation coefficient between the lipid contents in whole serum and each lipoprotein fraction

Lipid	n	Whole serum and HDL		Whole serum and LDL		Whole serum and VLDL	
		r[a]	p[b]	r	p	r	p
Cholesterol ester	11	0.93	0.01	-0.57	NS[c]	-0.25	NS
Free cholesterol	11	0.48	NS	0.72	0.05	0.47	NS
Triglyceride	10	0.69	0.05	0.95	0.01	0.92	0.01
Phosphatidyl choline	11	0.87	0.01	-0.65	0.01	0.03	NS
Sphingomyelin	11	0.83	0.01	0.78	0.05	0.53	NS
Lysophosphatidyl choline	11	0.97	0.01	-0.72	0.05	0.19	NS

a) Correlation coefficient. b) Probability of significant correlation coefficient.
c) Not significant.

Table II-11 shows relationships between the lipid contents in erythrocyte membranes and each lipoprotein fraction. Free cholesterol content in erythrocyte membranes correlated positively with contents of cholesterol ester, free cholesterol, triglyceride, phosphatidyl choline and lysophosphatidyl choline content in LDL and triglyceride in VLDL. Erythrocyte phosphatidyl ethanolamine content correlated positively with the contents of cholesterol ester, free cholesterol, triglyceride, phosphatidyl choline and sphingomyelin in LDL and free cholesterol, triglyceride and sphingomyelin in VLDL. Erythrocyte sphingomyelin content correlated with contents of all the lipids in LDL and free cholesterol, triglyceride and sphingomyelin contents in VLDL.

Table II-11. Correlation coefficient between the lipid contents in erythrocyte membranes and each lipoprotein fraction

Lipoprotein	Lipid	n	Erythrocyte membrane lipid: Free cholesterol r[a]	Free cholesterol p<[b]	Phosphatidyl ethanolamine r	Phosphatidyl ethanolamine p<	Phosphatidyl serine r	Phosphatidyl serine p<	Phosphatidyl choline r	Phosphatidyl choline p<	Sphingomyelin r	Sphingomyelin p<
HDL	Cholesterol ester	11	-0.84	0.01	-0.61	0.05	-0.05	NS[c]	-0.45	NS	-0.61	0.05
	Free cholesterol	11	-0.40	NS	-0.13	NS	-0.12	NS	-0.36	NS	-0.07	NS
	Triglyceride	11	0.29	NS	0.20	NS	-0.59	NS	-0.01	NS	0.29	NS
	Phosphatidyl choline	11	-0.92	0.01	-0.57	NS	-0.14	NS	-0.38	NS	-0.61	0.05
	Sphingomyelin	11	-0.38	NS	0.03	NS	-0.22	NS	-0.20	NS	0.09	NS
	Lysophosphatidyl choline	11	-0.87	0.01	-0.78	0.01	0.19	NS	-0.26	NS	-0.81	0.01
LDL	Cholesterol ester	11	0.94	0.01	0.79	0.01	0.19	NS	0.42	NS	0.83	0.01
	Free cholesterol	11	0.91	0.01	0.80	0.01	0.00	NS	0.40	NS	0.91	0.01
	Triglyceride	11	0.72	0.05	0.66	0.05	-0.31	NS	0.25	NS	0.84	0.01
	Phosphatidyl choline	11	0.87	0.01	0.60	0.05	0.00	NS	0.42	NS	0.71	0.05
	Sphingomyelin	11	0.56	NS	0.76	0.01	-0.21	NS	0.31	NS	0.90	0.01
	Lysophosphatidyl choline	11	0.90	0.01	0.59	NS	0.06	NS	0.36	NS	0.71	0.05
VLDL	Cholesterol ester	11	0.33	NS	0.55	NS	0.06	NS	0.32	NS	0.50	NS
	Free cholesterol	11	0.53	NS	0.75	0.01	-0.08	NS	0.41	NS	0.73	0.05
	Triglyceride	10	0.63	0.05	0.65	0.05	-0.49	NS	0.23	NS	0.82	0.01
	Phosphatidyl choline	11	0.19	NS	0.32	NS	-0.06	NS	0.38	NS	0.23	NS
	Sphingomyelin	11	0.37	NS	0.61	0.05	-0.31	NS	0.29	NS	0.69	0.05
	Lysophosphatidyl choline	11	-0.36	NS	-0.18	NS	-0.22	NS	-0.05	NS	-0.33	NS

a) Correlation coefficient. b) Probability of significant correlation coefficient.
c) Not significant.

7) Other test results on intravascular hemolysis

Table II-12 shows results for intravascular hemolysis test. Serum total bile acid level in the CS group was higher than in the heartwrom-free group, but lower than in the serious group, in which severe intravascular hemolysis was not found. Serum osmolality level in the CS group was higher than in the heartworm-free and serious group. However, the levels were not so high, leading to the intravascular hemolysis directly, and did not change significantly after heartworm removal, at which intravascular hemolysis disappeared. Blood ATP level was not different between the heartworm-free and CS groups. Serum phosphatidyl choline and lysophosphatidyl choline levels were lower in the CS group. However, erythrocyte glycerophospholipids (phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl choline) levels tended to be higher in the serious group, but those in the CS group were not different from in the heartworm-free group. Direct Coomb's test was negative in all dogs examined.

Blood coagulation test results indicated decrease in platelet count and increase in factor VIII level in the CS group (Table II-13). Although a few dogs had abnormal values, levels in prothrombin time, activated partial thromboplastin time, fibrinogen concentration and fibrinogen-fibrin degradation products concentration were not statistically different between the heartworm-free and CS groups.

Table II-14 shows incidence of thrombi in the principal organs of 15 dogs with spontaneous CS. Whether

Table II-12. Other results of intravascular hemolysis tests

Item	Heartworm-free			Serious			CS			p<[b]
	n	Mean	S.D.[a]	n	Mean	S.D.	n	Mean	S.D.	
Serm total bile acid (ng/ml)	94	5.3 (1 - 14)	3.9	14	38.0 (9 - 155)	40.8**	26	24.4 (3 - 49)	18.3**	0.01
Serum osmolality (mOsm/kg)	43	296 (286 - 306)	5	16	289 (262 - 307)	12	40	310 (272 - 370)	24**	0.01
Blood ATP (μmol/gHb)	25	1.93 (1.63 - 2.81)	0.22		ND		12	2.11 (0.90 - 3.49)	0.83	-
Serum phosphatidyl choline (mg/dl)	18	348 (157 - 434)	83	12	287 (78 - 453)	110	17	183 (46 - 428)	120**	0.01
Serum lysophosphatidyl choline (mg/dl)	18	25 (10 - 36)	7	12	18 (6 - 28)	6**	17	17 (0 - 32)	9**	NS
Erythrocyte phosphatidyl ethanolamine (mg/gHb)	18	2.76 (2.03 - 3.81)	0.84	12	4.15 (0.85 - 11.86)	2.77	18	2.64 (0.44 - 4.77)	1.35	NS
Erythrocyte phosphatidyl serine (mg/gHb)	18	1.42 (0.66 - 2.09)	0.51	12	2.16 (0.90 - 4.04)	0.78**	18	1.55 (0.48 - 2.86)	0.77	0.01
Erythrocyte phosphatidyl choline (mg/gHb)	18	7.39 (6.12 - 9.21)	1.51	12	9.15 (6.06 - 16.76)	3.22	18	7.56 (4.88 - 10.35)	1.47	NS

a) Standard deviation. b) Probability of significant difference between the serious and CS groups.
*, **: Each asterisk indicates probability of significant difference from the heartworm-free group at $p<0.05$ and $p<0.01$ level, respectively.

Table II-13. Blood coagulation test results

Item	Heartworm-free n	Mean	SD[a]	CS n	Mean	SD	p[b]
Platelet ($x10^4/\mu l$)	23	32.1	7.1	19	15.9	6.3	0.01
Prothrombin time (sec)	23	8.0	1.3	21	7.9	1.6	NS[c]
Activated partial thromboplastin time (sec)	23	15.2	1.0	21	17.1	6.1	NS
Fibrinogen (mg/dl)	23	205	39	21	238	131	NS
Factor VIII (%)	23	102	18	16	141	71	0.05
Fibrinogen-fibrin degradation products ($\mu g/ml$)	23	≦5		18	5.6[d]	2.5	NS

a) Standard deviation. b) Probability of significant difference. c) Not signifcant.
d) Geometric mean.

before or after heartworm removal, death or recovery, and with or without high concentration of plasma hemoglobin, the thrombi were sporadically found in the large and middle vessels. However, the thrombi were not found in the capillary and glomerulus. Findings of slight activation of the blood

Table II-14. Incidence of thrombi in principal organs in dogs with spontaneous CS

Dog No.		93	94	144	145	195	197	259	227	228	230	244	251	250	288	21
Heartworm removal		ND[a]	ND	ND	ND	ND	ND	ND	ND	DN	DN	DN	DN	DN	DN	DN
Result		D[b]	D	D	S[d]	D	D	D	D	D	S	S	S	D	D	D
Lung	Large vessel	−	++	−	−	+	−	+	+	−	+	+	+	+	−	−
	Middle vessel	−	+	−	+	++	−	−	+	−	+	−	−	++	−	−
	Capillary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Liver	Large vessel	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
	Middle vessel	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	Capillary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kidney	Large vessel	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
	Middle vessel	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Capillary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Glomerulus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

a) Not done. b) Done. c) Dead. d) Sacrificed.

coagulation system were found, but consumptive coagulopathy were not observed. The occurrence of disseminated intravascular coagulation (DIC) could not be proved.

Discussion

Various mechanisms have been reported in intravascular hemolysis in dogs and man [22, 58]. The hemolysis in dogs with CS is acquired, but not congenital, and relationship of heartworms with the hemolysis is very close. Hemolytically active chemical substances such as bile acids, which had a detergent activity [24, 44], and phospholipids, of which the increase has been known as congenital high phosphatidyl choline hemolytic anemia [34, 51], were not related directly with hemolysis. Immunological hemolysis was also denied from negative results of Coombs' test and from no differences in circulating immune complexes and total hemolytic compliment between the dogs with and without CS [5]. As different from paroxysmal hemoglobinuria in calves [50], serum osmolality was not involved in intravascular hemolysis in CS. Moreover, the plasma of diseased dogs did not lyse erythrocytes of normal dogs. Therefore, the chemical, immunological and osmotic mechanisms did not contribute to increase in intravascular hemolysis in CS.

On the other hand, most dogs with CS show severe liver dysfunction, so the disease is also termed "liver failure syndrome". The LCAT is synthesized only in the liver, and the enzyme transfers fatty acids from 2-position of lecithin to

cholesterol in circulating plasma [18]. Since the injured liver might reduce LCAT synthesis, serum LCAT activity reduced in liver failure. Because of low enzyme activity, free cholesterol level in plasma increased, with cholesterol ester ratio decreasing. The free cholesterol is a major component of LDL, so plasma LDL concentration simultaneously increases. Free cholesterol in LDL and erythrocyte membranes exchanges freely [13, 15]. Therefore, increase of free cholesterol in LDL reflects in erythrocyte membranes. Erythrocyte membranes loaded excessive free cholesterol, which was expressed as the abnormal erythrocyte shapes having excess membranes such as acanthocytes and codocytes. Excess cholesterol loading makes to decrease elasticity of the eryhtrocyte membranes [14]. Therefore, the mechanical and osmotic fragilities of erythrocytes increased. Dogs with CS possessed serial changes from liver dysfunction to increase in erythrocyte mechanical fragility. It was considered that the circulating erythrocytes have the tendency to be injured easily with mechanical force in CS.

Fragmented erythrocytes observed in peripheral blood suggested presence of hemolysis by physical mechanism. As mentioned in the introduction, Kociba et al. [40] reported two cases with DIC and this syndrome, and suggestied that the hemolysis resulted from mechanical damage of erythrocytes by contact with intravascular fibrin strands which were fomed in microvessels. Dogs with CS had severe intravascular hemolysis, injuries of the organs such as the liver and kidney, and

vascular damage. These injuries might activate the blood coagulation and fibrinolytic systems, and the possibility to complicate DIC in CS might be large. However, typical findings of DIC such as elongation of coagulation time, consumption of plasma coagulation factors, and activation of fiblinolysis could not be found in 21 dogs with spontaneous CS. Therefore, it was concluded that the DIC was not the direct cause of intravascular hemolysis in CS.

In dogs with CS, heartworms presented at the tricuspid valve orifice between the right atrium and right ventricle. Following heartworm removal from their place, plasma hemoglobin levels decreased rapidly. In contrast, when heartworms occurred at the tricuspid valve orifice, plasma hemoglobin levels elevated [36]. It was sure that the worms locating from the right atrium to the right ventricle related directly with the increase in intravascular hemolysis in CS. Buoro and Atwell [10] noted the attention to heartworms in the right atrium, and considered that erythrocytes were destroyed there due to shearing force quoting from our data [25], because the right atrium is a low-pressure/high velocity chamber with little capacity for laminar flow. However, the right atrium is an expandable chamber. The blood flow volume might be able to maintain, even if the worms located in the right atrium. Moreover, the insertion of filaria-like vinyl strings into the right atrium and venae cavae could not reproduce severe intravascular hemolysis [42]. Also, in the present study, heartworm-like silicone tubes only in the right

atrium did not induce severe intravascular hemolysis.

Filarid worm echoes were observed at the tricuspid valve orifice projecting from the right atrium to the right ventricle at the diastolic phase and returning to the right atrium at the systolic phase and returning to the right atrium at the systolic phase in all dogs with naturally acquired CS. The bloodstream flow in the right heart is severely deranged with these heartworms, especially at the tricuspid valve orifice brings about stenosis and regurgitation, which are expressed as cardiac murmur, jugular pulse, venous congestion and elevation of the CVP. The stenosis and regurgitation are followed by jet stream, turbulence and disruptive flow. These disturbances were most severe near the tricuspid valve orifice, the narrowest part between the right atrium and right ventricle. Under these conditions of bloodstream flow, it is easy to consider that the erythrocytes tending to be injured easily will be readily destroyed by the shearing force and physical collision. This mechanism of intravascular hemolysis may be similar to that of traumatic cardiac hemolytic anemia in man [11, 46, 52].

In spontaneous cases, the degree of intravascular hemolysis varied. Some cases had low plasma hemoglobin levels, but others extremely higher levels. Also, in the experiment in which silicone tubes were inserted into the tricuspid valve orifice, the number and position of tubes related closely with plasma hemoglobin levels. These findings suggest that the degree of intravascular hemolysis may be influenced by the

condition of heartworms at the tricuspid valve orifice, because the abnormality of bloodstream flow and function of the tricuspid valve are mainly influenced by the heartworm condition there.

4. SECTION III. Factors inducing migration of heartworms from pulmonary arteries towards right atrium

Introduction

As shown in SECTIONS I and II, heartworms at the tricuspid valve orifice play the most important role in development of caval syndrome (CS). Because usual habitat of heartworms is in the pulmonary arteries [20, 59], it must be considered that heartworms migrate from the pulmonary arteries toward the right atrium for some reasons in dogs with CS. Some investigators suggested that the critically large number of heartworms (overcrowding of heartworms in the pulmonary arteries) might induce heartworm migration from the pulmonary arteries [33, 48]. Certainly, most dogs with CS harbor a large number of heartworms. However, as shown in section I, some dogs with CS harbor only a small number of heartworms. Thus, the parasitism of a large number of worms does not sufficiently explain the heartworm migration. Recently, the authors have noted development of CS in a small part (10.5 %) of heartworm-infected dogs administered milbemycin D [37], a macrolide prophylactic for heartworm infection [55, 56]. The dogs showed depression, pale and congestive color in the visible mucous membranes, and dullness of heart sound before migration of heartworms. From clinical findings on the episode, the author supposed that changes in hemodynamics might associate closely with heartworm migration.

In this section, in order to investigate the cause of heartworm migration from the pulmonary arteries, right heart hemodynamics are determined in dogs administered milbemycin D. Based on changes in cardiopulmonary values after oral

administration of milbemycin D, the author attempts to diminish right cardiac output by administration of a $beta_1$-blocker (metoprolol [1]) in heartworm-infected dogs, and examines whether or not the heartworms migrate from the pulmonary arteries. Besides, because pulmonary arterial embolisms including dead heartworms are frequently found in dogs with spontaneous CS as shown in section I, dead heartworms are inserted into the pulmonary arteries in order to alter hemodynamics. Then, in order to examine the effects of physical obstruction of the pulmonary arteries and release of body fluid from the dead heartworms into the circulation, heartworm-like silicone tubes are inserted into the pulmonary arteries, and the body fluid of heartworms is infused intravenously, respectively. Then, changes in right heart hemodynamics and heartworm migration are investigated.

Materials and methods

1) Heartworm migration induced by administration of milbemycin D

Six mongrel dogs, 3 to 10 years old 6 to 13.5 kg body weight, were used. These dogs were heartworm-infected and circulating microfilaria positive. Dogs were administered milbemycin D (Sankyo Co. Ltd., Tokyo) at a dose of 1.5 mg/kg body weight intra-gastrically with a tube.

Two-dimensional echocardiography was done with a ultrasonic convex array scanner (EUB-40, Hitachi Medical Corp., Tokyo). Right heart hemodynamics were determined under

general anesthesia with a drip infusion of 0.2 % ketamine hydrochloride (Ketalar, Sankyo Co. Ltd., Tokyo). Blood pressures in the pulmonary artery and right cardiac output were determined by the same methods described in section I.

2) Heartworm migration induced by administration of $beta_1$-blocker (metoprolol)

Six dogs were used in the experiment. Dogs were confirmed to suffer from heartworm parasitism only in the pulmonary arteries by two-dimensional echocardiography. The animals were pre-medicated with diazepam (Cercine, Takeda Chemical Industries, Ltd., Osaka) at a dose rate of 0.5 to 0.8 mg/kg body weight and anesthetized with a drip infusion of 0.2 % ketamine hydrochrolide. A $beta_1$-blocker, metoprolol tartrate (Seloken, Fujisawa Pharmaceutical Co. Ltd., Tokyo), was administered to dogs intra-gastrically at a dose rate of 8 mg/kg body weight. To examine effects of anesthesia alone, hemodynamic parameters were measured in 2 dogs under the same anesthesia without metoprolol treatment. One week after control meausrements, these 2 dogs were treated with metoprolol like as the other 4 experimental dogs. Two-dimensional echocardiography and measurements of cardiopulmonary function values were done by the same methods described in section I.

3) Heartworm migration associated with death of heartworms

Dead heartworms were inserted into the pulmonary arteries through the jugular vein in 14 heartworm-infected dogs under general anesthesia. Heartworms removed from the other dogs with a flexible alligator forceps, were killed by freezing, and stored at -30 °C till the insertion. The number of dead worms inserted was from 6 to 38. After insertion of dead heartworms, clinical signs of the dogs were observed carefully. Heartworm migration was confirmed by auscultation of characteristic systolic heart murmur and presence of heartworms at the tricuspid valve orifice by use of two-dimensional echocardiography.

From 5 to 10 silicone tubes (M-Silico, No. 00, Shin-Etsu Polymer Co. Ltd., tokyo), 0.5 mm in diameter and 250 mm in length, were inserted into the pulmonary arteries of 12 dogs infected with heartworms. Cardiopulmonary function values were determined before and 1, 3, 7 and 9 days after insertion of silicone tubes in 8 dogs.

The body fluid of heartworms was prepared as follows; ten female worms were killed by freezing at -30 °C. Worms were cut into small fragments with scissors in 20 ml of 5 % glucose solution. The solution was shaken and centrifugated at 12000 rpm for 30 minutes. The supernatant was used as body-fluid extract. Two ml of solution, which included body fluid to equivalent with one female worm, was infused intravenously in 3 heartworm-infected dogs. Cardiopulmonary function values and systemic blood pressure were determined under general anesthesia with a drip infusion of 3 % ketamine hydrochloride

solution.

Two-dimensional echocardiography and determination of cardiopulmonary function values were done with the same procedures described in SECTION I. Systemic blood pressure was measured by the oscillometric method (BP-203NP, Nippon Colin Co. Ltd., Komaki, Aichi).

Results

1) Heartworm migration induced by administration of milbemycin D

As shown in Table III-1, the number of adult heartworms harboring in experimental dogs was 20 to 53, and the number of worms per kg body weight was 2.2 to 4.5. Heartworm migration from the pulmonary arteries to the tricuspid valve orifice and right atrium was observed at 50 to 105 minutes after drug administration in 4 of 6 dogs. On

Table III-1. Heartworm migration after milbemycin D administration and number of heartworms harbored

Dog No.	Heartworm migration	Dog			No. of adult heartworms	No. of worms/ kg body weight
		Age (y)	Sex	BW (kg)		
1036	+	3	Male	9.0	20	2.2
1039	+	5	Male	6.0	20	3.3
1148	+	10	Male	9.0	27	3.0
1152	+	4	Male	10.0	45	4.5
1081	−	4	Female	6.5	22	3.4
1129	−	5	Female	13.5	53	3.9

Fig. III-1. B-mode echocardiogram at the time of onset after administration of milbemycin D. RV: Right ventricle, LV: Left ventricle, TV: Tricuspid valve, RA: Right atrium, Arrow: Heartworm echo.

echocardiogram, heartworm echoes same as natural cases were observed at the tricuspid valve orifice (Fig. III-1). Ventricular and atrial premature heartbeats appeared simultaneously with heartworm migration (Fig. III-2). Coinciding with recognition of heartworm echoes at the tricuspid valve orifice, systolic cardiac murmur was audible. Changes in systolic right ventricular pressure after milbemycin D administration are shown in Fig. III-3. The pressure rose strikingly in one dog (No. 1129), but heartworms did not migrate. Another heartworm-non-migration case (No. 1081) showed drifting changes in systolic right ventricular pressure. In 4 heartworm-migration cases, the pressure did not display uniform changes; reight ventricular pressure elevated

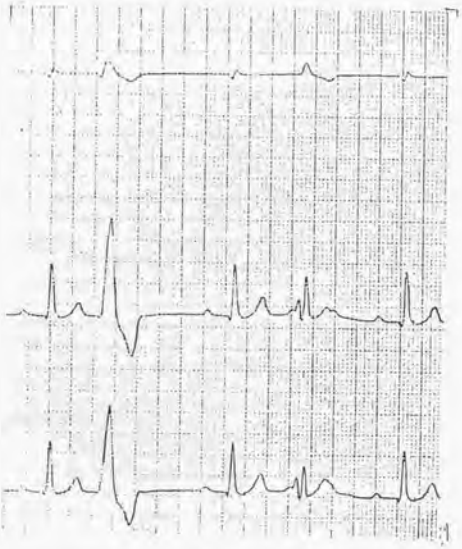

Fig. III-2. Electrocardiogram at the time of heartworm migration

gradually and subsequently fell in one dog (No. 1036), decreased slightly in one dog (No. 1039), and dropped but subsequently rose in two dogs (Nos. 1148 and 1152). Cardiac output did not change in heartworm-non-migration cases (Fig. III-4). In all dogs with heartworm migration, however, cardiac output decreased clearly before heartworm migration. Cardiac output fell from 46.7 % to 69.3 % of the pre-administration level. After administration of milbemycin D, heart rate increased transiently in 5 of 6 dogs, and decreased gradually.

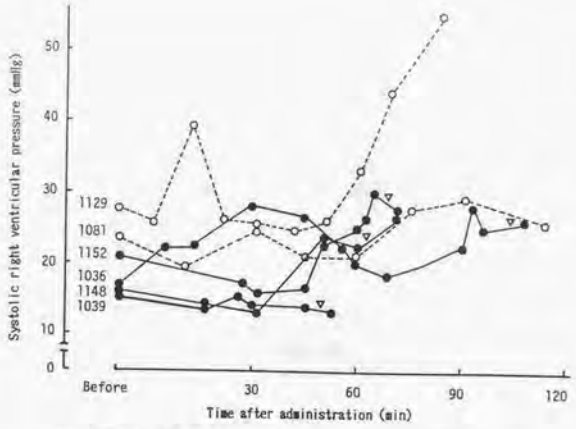

Fig. III-3. Changes in systolic right ventricular pressure after administration of milbemycin D
○--○: Heartworm-non-migration case.
●—●: Heartworm-migration case.
▽: Heartworm migration.

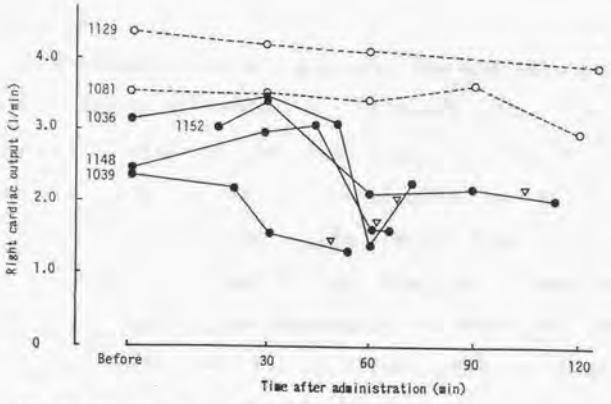

Fig. III-4. Changes in right cardiac output after administration of milbemycin D
○--○: Heartworm-non-migration case.
●—●: Heartworm-migration case.
▽: Heartworm migration.

Table III-2. Changes in other cardiovascular values before milbemycin D administration and before heartworm migration

Item	Group	n	Before administration Mean	SD	Before heartmorm migration[a] Mean	SD
Heart rate (beat/min)	Migration	4	118	16	133	26
	Non-migration	2	145	15	111	23
Stroke volume (ml/beat)	Migration	4	23.3	2.2	12.3	2.0
	Non-migration	2	28.3	7.9	31.1	10.6
Cardiac index (ml/min/kg)	Migration	4	331	54	194	39
	Non-migration	2	435	154	376	123
Stroke index (ml/beat/kg)	Migration	4	3.25	0.55	1.53	0.53
	Non-migration	2	3.01	0.70	2.85	0.82

a) 90 minutes after administration of milbemycin D.

Heart rate was slightly higher in heartworm-migration cases immediately before heartworm migration (Table III-2). Stroke volume, cardiac index and stroke index also decreased before heartworm migration in all dogs.

2) Heartworm migration induced by administration of $beta_1$-blocker

Heartworms did not migrate from the pulmonary arteries in 2 dogs during the ketamine anesthesia without $beta_1$-blocker (metoprolol) treatment. Besides, from 40 to 125 minutes after metoprolol administration, heartworms migrated from the pulmonary arteries toward the right atrium in all 6 dogs (Figs. III-5, III-6 and III-7). Heartworms remained at the tricuspid valve orifice throughout the experiment in 2 dogs (Nos. 1280 and 1301-II), but the worms returned to the pulmonary arteries from 5 to 10 minutes after migration in 3 dogs (Nos. 1275, 1276 and 1295-II). In another dog (No. 1323), some heartworms hurdled the pulmonary valve transiently at 35

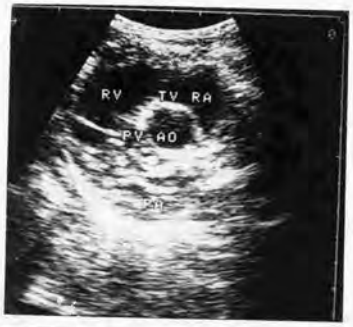

Fig. III-5. B-mode echocardiogram before administration of metoprolol. Heartworms are observed only in the pulmonary arteries. Rv: Right ventricle, TV: Tricuspid valve, RA: Right atrium, AO: Aorta, PA: Pulmonary artery.

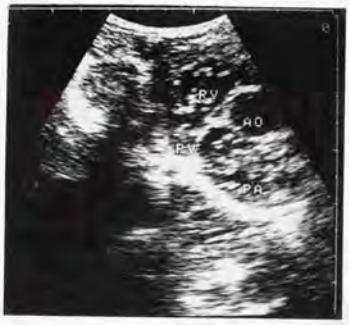

Fig. III-6. B-mode echocardiogram at the time of heartworm migration. Heartworms migrate from the pulmonary arteries to the right ventricle. RV: Right ventricle, AO: Aorta, PV: Pulmonary valve, PA: Pulmonary artery.

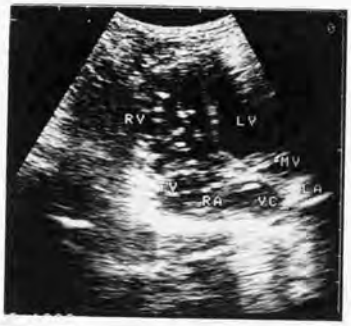

Fig. III-7. B-mode echocardiogram at the time of onset. Heartworms move reciprocatedly between the right atrium and right ventricle. RV: Right ventricle, LV: Left ventricle, TV: Tricuspid valve, MV: Mitral valve, RA: Right atrium, LA: Left atrium, VC: Venae cavae.

minutes after metoprolol administration, but did not reach the right atrium.

Heart rate (Fig. III-8) decreased in all dogs after metoprolol administration, but transiently decreased or did not decrease in dogs without metoprolol treatment. Right cardiac output (Fig. III-9) increased in 2 dogs during ketamine anesthesia without metoprolol treatment, but decreased in all dogs receiving metoprolol. Stroke volume, as shown in Fig. III-10, also increased in dogs without the treatment. After metoprolol administration, stroke volume did not change in 2 dogs (Nos. 1276 and 1295-II), increased, but

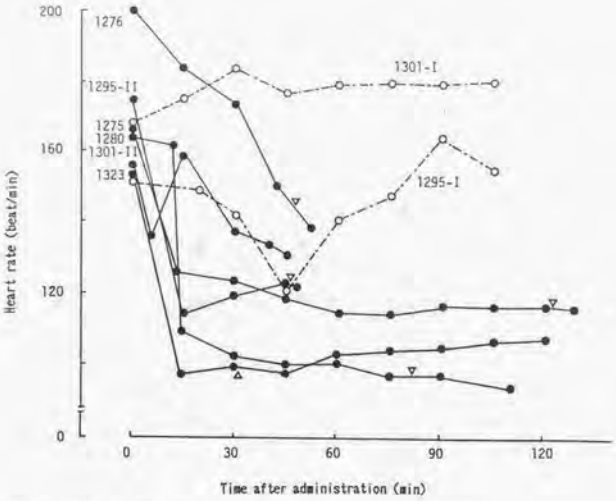

Fig. III-8. Changes in heart rate after administration of metoprolol
●—●: Heartworm-migration case.
○···○: Case without metoprolol treatment.
▽: Heartworm migration.

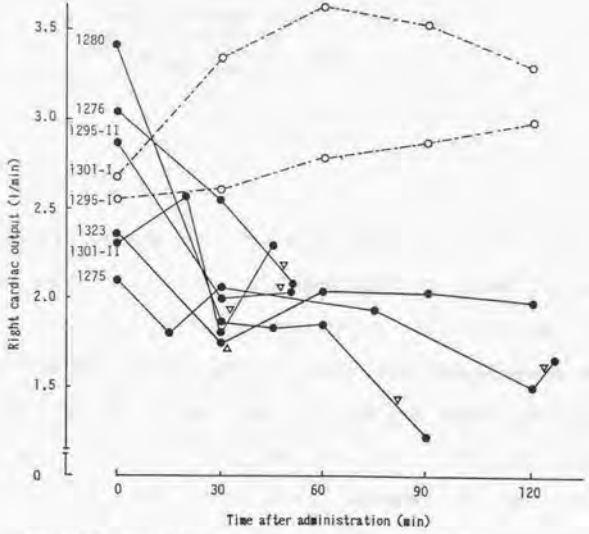

Fig. III-9. Changes in right cardiac output after administration of metoprolol
●—●: Heartworm-migration case.
○···○: Case without metoprolol treatment.
▽: Heartworm migration.

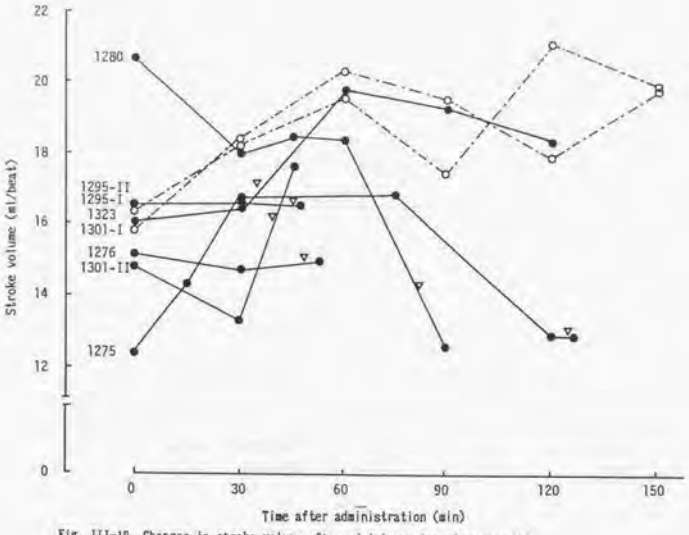

Fig. III-10. Changes in stroke volume after administration of metoprolol
●—●: Heartworm-migration case.
○—○: Case without metoprolol treatment.
▽: Heartworm migration.

then returned to the pre-treatment level in 1 dog (No. 1275), decreased in 2 dogs (Nos. 1280 and 1301-II), and increased in 1 dog (No. 1323). In 3 dogs, in which stroke volume did not changed from the pre-treatment level when heartworms migrated, heartworms stayed in the right atrium for short periods (5 to 10 minutes), then the worms returned to the pulmonary arteries. In 2 dogs in which stroke volume decreased, heartworms stayed in the right atrium and tricuspid valve orifice throughout the experiment. In one dog with increased

stroke volume, heartworms hurdled the pulmonary valve, but did not reach the right atrium. Systolic right ventricular pressure elevated in 1 dog (No. 1295-II), fell in 3 dogs (Nos. 1275, 1276 and 1280), and did not change in 2 dogs (Nos. 1301-II and 1323) after metoprolol administration (Table III-3). The pressure was within the normal level during the experiment in 1 dog (No. 1323), in which the worms hurdled the pulmonary valve transiently.

Table III-3. Changes in systolic pulmonary arterial pressures after β_1-blocker (metoprolol) administration

Item	Dog No.	Metoprolol treatment	Heartworm migration	Before administration[a]	Before worm migration[b]
	1295-I	−	−	30.6	39.5
	1301-I	−	−	42.2	47.3
Systolic right ventricular pressure (mmHg)	1275	+	+	61.7	43.7
	1276	+	+	51.9	39.0
	1280	+	+	59.1	49.3
	1295-II	+	+	31.5	42.3
	1301-II	+	+	35.3	39.2
	1323	+	−	24.1	24.1

a) Before administration of metoprolol. b) Immediately before heartworm migration (in cases without migration, the data 60 min after treatment are shown).

Numbers of adult heartworms counted at necropsy are shown in Table III-4. Five dogs, in which heartworms migrated to the right atrium, harbored a relatively large number of heartworms from 38 to 110. The number of heartworms per kg body weight ranged from 3.50 to 10.00. On the other hand, one dog, in which the worms did not migrate to the right atrium,

harbored a small number of worms (15 worms and 1.67 worms/kg body weight).

Table III-4. Heartworm migration induced by the administration of β_1-blocker (metoprolol) and number of heartworms harbored

Dog No.	Heartworm migration	Dog				No. of adult heartworms	No. of worms/kg body weight
		Breed	Sex	Age (y)	BW (kg)		
1275	+	Mongrel	Male	5	10.0	68	6.80
1276	+	Mongrel	Female	6	8.0	38	4.75
1280	+	Mongrel	Male	5	13.0	78	6.00
1295	+	Mongrel	Female	8	11.0	110	10.00
1301	+	Collie	Female	10	12.0	42	3.50
1323	±[a]	Mongrel	Female	4	9.0	15	1.67

a) Heartworms hurdled the pulmonary valve, but did not reach the right atrium.

3) Heartworm migration associated with death of heartworms

As shown in the previous section, embolization of the pulmonary arteries with dead heartworms were observed in almost all dogs with spontaneous CS. Then, in order to investigate whether heartworms migrated from the pulmonary arteries, dead heartworms were inserted into the pulmonary arteries of heartworm-infected dogs. Table III-5 shows the heartworm migration after dead-worm insertion and numbers of dead heartworms inserted and live heartworms harboring in the right heart of dogs. After insertion of dead heartworms into the pulmonary arteries, live worms migrated from the pulmonary arteries to the right atrium and tricuspid valve orifice in 10

Table III-5. Heartworm migration after insertion of dead worms and numbers of dead heartworms inserted and live heartworms

Dog No.	Heartworm migration	Period after insertion	No. of dead worms inserted	No. of live worms harbored
1617	+	3 days	10 (10[a], 0[b])	73 (33, 40)
1622	+	IM[c]	38 (19, 19)	35 (19, 16)
1625	+	1 day	20 (15, 5)	51 (33, 18)
1628	+	7 days	25 (10, 15)	39 (26, 13)
1631	+	IM	14 (10, 4)	64 (33, 31)
1634	+	IM	20 (20, 0)	42 (28, 14)
1635	+	IM	20 (20, 0)	70 (38, 32)
1685	+	2 days	17 (11, 6)	71 (41, 30)
1706	+	IM	15 (15, 0)	55 (36, 19)
1711	+	IM	10 (10, 0)	32 (19, 13)
1627	−	−	20 (18, 2)	42 (30, 12)
1704	−	−	25 (25, 0)	52 (34, 18)
1712	−	−	6 (6, 0)	19 (13, 6)
1789	−	−	8 (8, 0)	16 (8, 8)

a) Number of female worms. b) Number of male worms. c) Immediately or a few hours after insertion.

of 15 dogs. The characteristic heartworm echoes were observed at the tricuspid valve orifice on echocardiogram, and typical cardiac murmur was audible by auscultation in dogs with heartworm migration. Heartworm migration was observed immediately or within 3 hours after insertion in 6 dogs (early group), and 2 to 7 days after insertion in 4 dogs (late group). Heartworm migration did not observed in 4 dogs (non-migration group). Number of dead heartworms inserted was 7 to 25, and number of live worms residing was 8 to 73. There were no significant relationships among the heartworm migration, periods from insertion to migration, the number of dead heartworms inserted and the number of live worms resided. In Table III-6, cardiopulmonary function values after

Table III-8. Cardio-pulmonary values after insertion of dead heartworms

Item	Group	Dog No.	Before	Immediately[a]	1 day	3 days	7 days	9 days
Heart rate (beat/min)	Early	1706	161.5	138.2				
		1711	250.0	128.1				
	Late	1628	172.5	—	150.0	158.8	132.6	
		1728	135.0	—	122.2	135.9		
	Non-migration	1627	143.5	—	164.5	183.3	171.4	170.7
		1681	153.5	—	147.2	137.7	135.0	
		1704	130.4	—	—	130.8	—	125.8
		1789	157.4	—	156.6	143.5	141.8	186.7
Systolic pulmonary arterial pressure (mmHg)	Early	1706	37.6	22.5				
		1711	29.7	12.2				
	Late	1628	60.9	—	79.1	72.3	48.0	
		1728	35.1	—	37.6	51.6		
	Non-migration	1627	32.2	—	35.9	38.2	35.6	42.4
		1681	35.9	—	32.5	33.2	41.4	
		1704	30.3	—	—	32.2	—	27.0
		1789	39.8	—	44.9	32.2	32.3	41.8
Enddiastolic right ventricular pressure (mmHg)	Early	1706						
		1711	ND					
	Late	1628	0.9	4.8	4.5	0.4		
		1728	2.9	5.6	15.2			
	Non-migration	1627	0.6	-0.5	0.2	0.9	0.0	
		1681	3.2	4.4	4.6	3.9		
		1704	2.5	—	0.4	—	-0.1	
		1789	2.9	1.1	-0.8	0.3	0.7	
Right cardiac output (l/min)	Early	1706	2.66	UM[b]				
		1711	3.04	UM				
	Late	1628	2.99	—	2.67	2.84	1.89	
		1728	3.03	—	2.26	2.17		
	Non-migration	1627	3.49	—	2.55	2.43	2.22	2.76
		1681	2.96	—	1.70	1.91	2.67	
		1704	3.15	—	—	2.65	—	2.72
		1789	3.62	—	3.14	2.23	2.30	3.02
Stroke volume (ml/beat)	Early	1706	16.5	—				
		1711	12.2	—				
	Late	1628	17.3	—	17.8	17.9	14.2	
		1728	22.4	—	18.5	15.9		
	Non-migration	1627	24.3	—	15.5	13.3	12.9	16.2
		1681	19.3	—	11.6	13.9	19.8	
		1704	24.2	—	—	20.3	—	21.6
		1789	23.0	—	20.2	15.5	16.2	16.2
Total pulmonary resistance (dyns·sec·cm^{-5}·kg)	Early	1706	11038					
		1711	3333					
	Late	1628	10351	13606	12140	12622		
		1728	6012	8054	11370			
	Non-migration	1627	6031	9381	9880	9883	7236	
		1681	3324	6420	5238	4526		
		1704	7029	—	8516	—	8477	
		1789	4585	5905	6084	5865	5771	

a) Time after insertion. b) Unmeasurably lower level (<1.0 l/min).

insertion of dead heartworms are presented. Heart rate decreased in dogs of the early group, but did not show the uniform changes in dogs of the late and non-migration groups. Systolic pulmonary arterial pressure fell immediately after insertion in the early group. Pulmonary arterial pressure elevated in the late group, but did not change in the non-migration group. Endodiastolic right ventricular pressure did not change in a dog of early group. The endodiastolic pressure elevated in the late group, but did not change in the non-migration group. Right cardiac output decreased to an unmeasurably low level (<1.0 l/min) in the early group, and decreased slightly also in the late and non-migration groups. Stroke volume could not be calculated in the early group, because right cardiac output was unmeasurably low. Stroke volume decreased in the late group, but showed drifting changes in the non-migration group. Total pulmonary resistance could not be calculated also in the early group. Pulmonary resistance decreased in the late group, but showed drifting changes in the non-migration group. Total pulmonary resistance could not be calculated also in the early group, and did not change in the non-migration group.

The data suggested that there might be two factors in migration of live heartworms after insertion of dead heartworms. The period for heartworm migration after insertion was very short, and cardiopulmonary function values indicated reduced venous return blood volume or diminished pumping action of the heart in the early group. Elevation in pulmonary

arterial pressure and increase in total pulmonary resistance indicated increased after-load of the right heart in the late group. There were no apparent changes in cardiopulmonary values of the non-migration group.

Then, instead of dead heartworms, heartworm-like silicone tubes were inserted into the pulmonary arteries of heartworm-infected dogs in order to examine the effect of physical obstruction of the pulmonary arteries. From 1 to 11 days after tube insertion, live heartworms migrated from the pulmonary arteries toward the right atrium in 4 of 12 cases (Table III-7). There were no cases in which heartworms

Table III-7. Heartworm migration after insertion of silicone tubes into the pulmonary arteries and numbers of tubes inserted and live heartworms harbored

Dog No.	Heartworm migration	Day after insertion	Dog			No. of tubes inserted	No. of live heartworms
			Age (y)	Sex	BW (kg)		
1718	+	8	3	Female	6.3	7	30 (20[a], 10[b])
1774	+	11	4	Male	12.5	10	12 (9, 3)
1757	+	1	6	Male	6.0	10	14 (9, 5)
1768	+	1	3	Female	7.5	5	61 (26, 35)
1712	−	−	2	Female	8.0	7	19 (13, 6)
1746	−	−	3	Female	9.0	5	28 (4, 14)
1727	−	−	3	Male	7.0	5	5 (2, 3)
1765	−	−	4	Female	9.0	8	10 (6, 4)
1785	−	−	10	Female	16.0	10	30 (15, 15)
1791	−	−	10	Male	12.8	12	8 (4, 4)
1795	−	−	3	Female	6.8	11	9 (8, 1)

a) Female. b) Male.

migrated from the pulmonary arteries immediately after tube insertion. Heart rate decreased after insertion in heartworm-migration cases (Table III-8). Systolic pulmonary arterial

Table III-8. Cardio-pulmonary values before and after insertion of heartworm-like silicone tubes into pulmonary arteries

Item	Dog No.	Before[a]	Days after insertion			
			1	3	7	9
Heart rate (beat/min)	1718	188	154	162	169	-
	1774	151	137	136	132	118
	Non-migration (n=6)	135[b]	144	133	144	144[d]
		10[c]	12	22	28	8
Systolic pulmonary arterial pressure (mmHg)	1718	37.2	40.0	43.9	32.7	-
	1774	37.6	46.1	46.3	45.5	51.2
	Non-migration (n=6)	26.1	29.7	27.2	29.7	27.4
		4.2	5.0	4.5	6.2	7.4
Endodiastolic right ventricular pressure (mmHg)	1718	2.0	4.0	3.2	-2.3	-
	1774	-0.83	2.2	2.6	4.1	5.2
	Non-migration (n=6)	1.8	2.5	3.3	1.7	0.5
		3.0	2.3	3.7	1.1	1.5
Right cardiac output (l/min)	1718	2.79	2.17	2.46	1.61	-
	1774	2.50	2.48	2.45	2.09	2.00
	Non-migration (n=6)	2.52	2.34	2.02	2.18	2.19
		0.31	0.45	0.29	0.24	0.12
Stroke volume (ml/beat)	1718	14.9	14.1	15.2	9.5	-
	1774	16.5	18.1	18.0	15.9	16.9
	Non-migration (n=6)	18.7	16.4	16.1	15.5	15.3
		2.3	4.3	4.6	3.1	1.3
Total pulmonary resistance (dyns·sec·cm^{-5}·kg)	1718	4748	6196	5669	7437	-
	1774	9087	11102	11238	13316	14540
	Non-migration (n=6)	5598	7521	7203	7178	6530
		1879	2881	1864	1422	1089

a) Before insertion. b) Mean. c) Standard deviation. d) n=4.

pressure and endodiastolic right ventricular pressure elevated after tube insertion in heartworm-migration cases. Right cardiac output decreased before heartworm migration in all 2 dogs. However, stroke volume decreased in one dog (No. 1718), but not in another (No. 1774) before heartworm migration. In heartworm-non-migration cases, cardiac output and stroke volume decreased after insertion of silicone tubes. Total pulmonary resistance increased after tube insertion in heartworm-migration cases. The resistance elevated slightly in heartworm-non-migration cases. Right cardiac output decreased, and pulmonary arterial pressure elevated, so total pulmonary resistance increased in migration cases. Cardiac output decreased, but pulmonary arterial pressure did not elevate in non-migration cases.

As another experiment, the body fluid of a female heartworm was infused intravenously into dogs with heartworm infection in order to investigate the effect of dead heartworms other than the physical obstruction (Table III-9).

Table III-9. Heartworm migration after infusion of body fluid of heartworms intravenously and numbers of tubes inserted and live heartworms harbored

Dog No.	Heartworm migration	Minute after infusion	Dog			No. of live heartworms
			Age (y)	Sex	BW (kg)	
1781	+	8	3	Female	4.7	5 (1[a], 4[b])
1791	+	11	10	Male	13.0	8 (4, 4)
1792	+	1	5	Male	17.6	24 (7, 17)

a) Females. b) Males.

Heartworms migrated from the pulmonary arteries to the right atrium from 4 to 7 minutes after infusion of the fluid, and worms reached the right atrium from 5 to 25 minutes after infusion in all 3 dogs. Immediately after infusion of heartworm body fluid, visible mucous membranes of dogs changed to pale color, and body surface feeled to be cold. Deep respiration was observed, and heart sound changed to dullness. The systolic cardiac murmur was audible following migration of heartworms. Systemic blood pressure fell promptly to below 60 mmHg, and maintained low levels (Fig. III-11). In 2 dogs (Nos. 1781 and 1792), systemic pressure fell to an

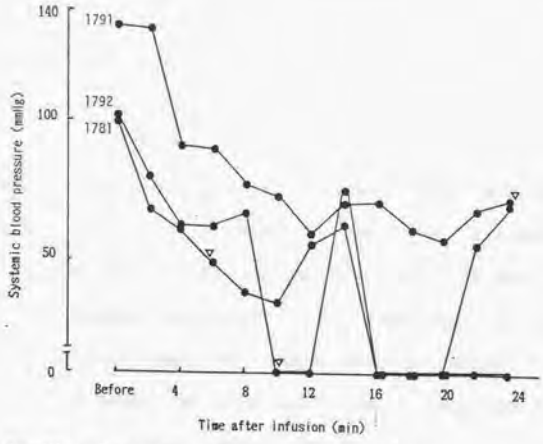

Fig. III-11. Changes in systemic blood pressure after infusion of heartworm-body fluid intravenously
▽: Heartworm migration.

Table III-10. Cardiopulmonary values after administration of body fluid of heartworms

Parameter	Dog. No.	Before administration	After administration		
			Immediately	5 min	15 min
Heart rate (beat/min)	1781	160	83	81	
	1791	132	155	173	140
	1792	97	61	ND[a]	
Systolic pulmonary arterial pressure (mmHg)	1781	40.6	24.8	17.2	
	1791	39.3	28.6	22.9	ND
	1792	32.4	26.8	ND	
Right cardiac output (l/min)	1781	1.50	UM[b]	UM	
	1791	2.63	2.52	1.13	1.06
	1792	3.77	1.61	UM	
Stroke volume (ml/beat)	1781	9.38	UM	UM	
	1791	19.91	16.24	6.52	7.57
	1792	38.80	26.22	UM	

a) Not done. b) Unmeasurably low level (<1.0 l/min).

unmeasurable level (Below 20 mmHg). As shown in Table III-10, heart rate, systolic pulmonary arterial pressure, right cardiac output and stroke volume decreased in all dogs after infusion of heartworm body fluid. Changes in heart rate, pulmonary arterial pressure, right cardiac output, and stroke volume were almost the same as those in the early group in which dead heartworms were inserted into the pulmonary arteries.

Discussion

It was demonstrated in this study that the decrease in right cardiac output should induce heartworm migration from the pulmonary arteries toward the right atrium. Decrease in right cardiac output implies decrease in blood flow volume and velocity in the pulmonary arteries. Heartworms are staying in the pulmonary arteries against the bloodstream flow in dogs with pulmonary dirofilariasis. When the blood-stream flow in the right heart system decreases, heartworms may migrate from the pulmonary arteries towards the right atrium. However, the degree of decrease in cardiac output for heartworm migration was different in individual dog. Dogs with heartworm disease harbored various number of mature and immature live heartworms, and had various degrees and many different kinds of vascular and parenchymal lesions in the lung. Volume and velocity of blood flow in the pulmonary arteries and ability of cardiopulmonary compensation may be different in individual dog, so that the threshold of cardiac output for heartworm migration may be various.

It was suggested that the decrease in right cardiac output after oral administration of milbemycin D was induced by decrease in venous blood return following the shock-like reaction. The shock-like reaction after milbemycin D administration was considered that the release of substances released from microfilariae might be associated [37, 38]. Metoprolol, an anti-hypertensive agent with cardio-selective $beta_1$-adrenoceptor blocking action ($beta_1$-blocker), make to

decrease heart rate and contractibility of the heart muscle [1, 21]. Right cardiac output thus decreased, and heartworm migration might be induced after milbemycin D administration and metoprolol administration.

Decrease in cardiac output by insertion of dead heartworms into the pulmonary arteries might involve 2 mechanisms; decrease in venous return and increase in total pulmonary resistance. When heartworms dies in the pulmonary arteries, the body fluid (or antigen) shall be released into circulation from heartworms. When the body fluid of heartworms was infused intravenously into dogs, not only heartworm-infected dogs but also heartworm-free dogs showed severe shock-like reaction (unpublished data). Also in the present study, dogs showed signs of shock-like reaction such as low blood pressure, peripheral vasoconstriction, low body temperature, dullness of heart sound and hemoconcentration. The shock-like reaction brings about decrease in venous return, so cardiac output decreased, and heartworms might migrate from the pulmonary arteries. Heartworm migration was induced by administration of body fluid equivalent to only one worm. Heartworm migration induced by infusion of heartworm body fluid may explain the sudden onset of spontaneous CS.

On the other hand, death of heartworms in the pulmonary arteries (insertion of dead heartworms) caused the obstruction with dead heartworms and/or with thrombi formated aroud dead heartworms. The episode of thromboembolism of the pulmonary arteries and heartworm migration could be reproduced

without effects of heartworm fluid by insertion of heartworm-like silicone tubes into the pulmonary arteries. Because of increased total pulmonary resistance (increased after-load of the right heart), pulmonary arterial pressure elevated, then right cardiac output decreased. Residing of a large number of heartworms also caused an elevation in the pulmonary arterial pressure and total pulmonary resistance resulting to cardiac output decrease. In dogs with naturally acquired CS, extremely severe pulmonary hypertension and a widely extended pulmonary arterial trunk indicated the presence of severe pulmonary thromboembolism. Actually, prunings of the pulmonary arteries were observed by angiography, and thromboembolism including dead heartworms at pruning positions were confirmed by autopsy in many cases. Increase in pulmonary vascular resistance with harboring of live heartworms and severe and wide-spread pulmonary thromboembolisms with dead heartworms reduced cardiac output, so that the heartworm migration may be induced.

From results mentioned above, heartworms may migrate from the pulmonary arteries toward the right atrium following decrease in cardiac output by one or more complex factors as follows; 1) increase of total pulmonary vascular resistance with residing a large number of heartworms, thromboembolism of the pulmonary arteries with dead heartworms and other pulmonary diseases with heartworm infection or other etiology, 2) decrease in venous return by decrease of circulating blood volume (shock-like reaction) and/or dilation of the blood vessels, and 3) decrease in pumping ability of the heart by

injuries of the valves and/or heart muscles and depression of cardiac function mediated by the dominated nerves.

5. DISCUSSION

The proposed pathophysiology of CS is summarized in Fig. D-1. In dogs with CS, heartworms locate at the venae cavae, right atrium, tricuspid valve orifice and right ventricle in addition of the pulmonary arteries as presented here and described by other investigators [6, 31, 32]. Different from chronic serious pulmonary heartworm disease, in which heartworms locate only in the pulmonary arteries, violent symptoms occur suddenly in dogs with CS, and most dogs die with acute course. Difference in symptoms between the CS and pulmonary heartworm disease may associate with difference

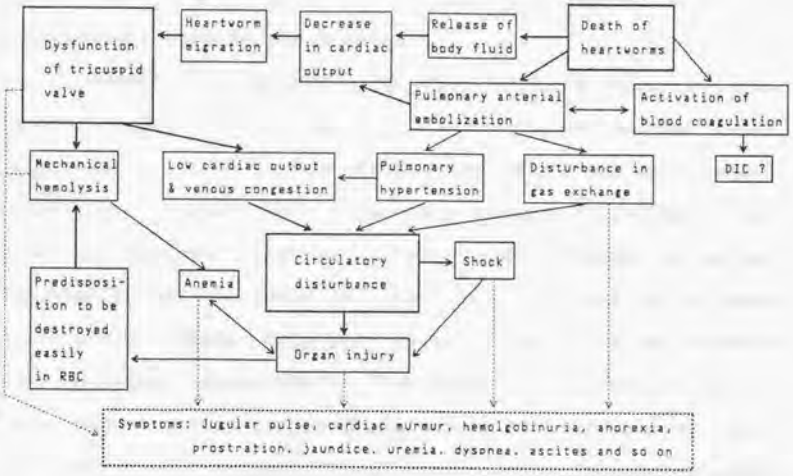

Fig. D-1 Proposed pathophysiology of CS.

in location of heartworms. Until now, it has been considered that the most important location of heartworms was at the venae cavae, as the disease was termed "caval syndrome or venae cavae syndrome" [30]. However, heartworms could be seen moving back and forth between the right atrium and right ventricle through the tricuspid valve orifice in all dogs with CS on echocardiograms as shown in the present study. Also, findings indicating regurgitation and stenosis at the tricuspid valve orifice, such as high a- and v-wave of right atrial pressure (high central venous pressure), and regurgitation of contrast medium from the right ventricle to the right atrium were clearly observed in dogs with naturally acquired and artificial CS. The parasitism of heartworms between the right atrium and right ventricle through the tricuspid valve orifice may play an important role in pathophysiology of the disease.

Presence of heartworms at the tricuspid valve orifice induces dysfunction of the valve. Following tricuspid valve dysfunction, right cardiac output was reduced, and venous congestion increased, then severe circulatory disturbance was raised up. Besides, pulmonary arterial embolization including dead heartworms were found in almost all dogs with CS as shown in the present study. Pulmonary arterial embolization induces severe pulmonary hypertension (increase in right-heart after-load) and disturbance in ventilation in the lungs, and also contributes to circulatory disturbance [45]. These processes are presented by fine allow in right-hand side of Fig. D-1.

The circulatory disturbance in CS might be induced by tricuspid valve dysfunction together with pulmonary arterial embolization. However, tricuspid valve dysfunction is characteristic in CS, because the process of circulatory disturbance following pulmonary arterial embolization presented also in dogs with pulmonary heartworm disease showing the signs of right heart failure (chronic serious heartworm disease).

Atwell [6] proposed that the entity of the disease was "shock". When circulatory disturbance is very severe, the dog may fall into "shock". However, it might be secondary from circulatory disturbance, because all dogs with CS did not show signs of shock such as collapse, low blood pressure and hypothermia. Symptoms in CS might result from tricuspid valve dysfunction (jugular pulse and cardiac murmur), mechanical intravascular hemolysis (anemia and hemoglobinuria), organ injury (jaundice, high serum enzyme activities and uremia), circulatory disturbance or shock (ascites, subcutaneous edema, anorexia and prostration) and hypoxemia (dyspnea, labored respiration and rough vesicular breath sound).

Heartworms at the tricuspid valve orifice also induce increase in intravascular hemolysis. As shown in Fig. D-2, following liver dysfunction including decrease in serum LCAT activity, circulating erythrocytes have acquired a predisposition to be destroyed easily with physical force resulting from serial changes in lipid contents of lipoproteins and erythrocyte membranes. Owing to heartworms at

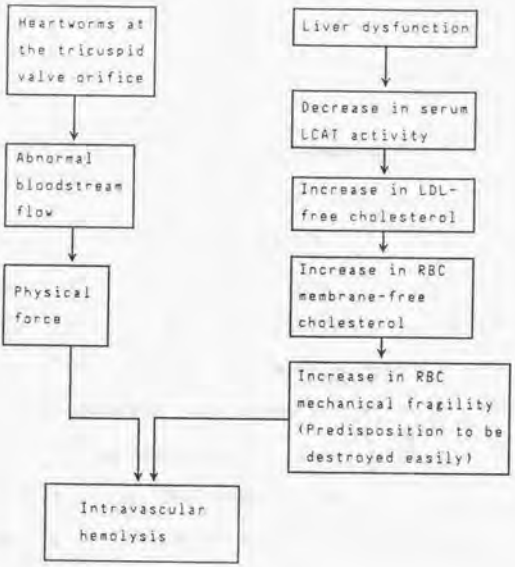

Fig. D-2. Possible mechanism of intravascular hemolysis in CS.

the tricuspid valve orifice, abnormal bloodstream flows such as jet stream, turbulence and disruption flows might be raised up near the tricuspid valve orifice. Under these abnormal conditions of bloodstream flow, erythrocytes were destroyed easily with shearing force of blood stream flow and physical collision for pericardium and/or surface of heartworms.

In dogs with naturally acquired CS, severity of symptoms varied in individual dog. Some dogs showed almost normal vigor and appetite in spite of presence of heartworms at the tricuspid valve orifice. Besides, a few worms induced

severe symptoms in some cases. Differences in symptoms may be associated with severity of abnormal bloodstream flow at the tricuspid valve orifice and disturbance of pulmonary circulation.

As mentioned above, heartworms at the tricuspid valve orifice play an important role in pathophysiology of CS. However, the cause of heartworm migration from the pulmonary arteries has not been elucidate. It was suggested that the parasitism of a large number of heartworms in the pulmonary arteries might induce heartworm migration towards the venae cavae [46]. However, as shown in section I, dogs residing a few number of heartworms also developed CS, then overcrowding of heartworms in the pulmonary arteries could not explain sufficiently the cause of heartworm migration. In the present study, heartworms migrated from the pulmonary arteries towards the right atrium when right cardiac output decreased. Cardiac output decreases for any of following reasons: increase in pulmonary vascular resistance, decrease in venous return and decrease in pumping ability of the heart [39]. In dogs with heartworm infection, increase in pulmonary vascular resistance resulted from harboring of live heartworms in the pulmonary arteries, pulmonary vascular thromboembolism and proliferation, and parenchymal lesions [45]. As shown in section III, insertion of dead heartworms and heartworm-like silicone tubes into the pulmonary arteries could induce decrease in cardiac output and migration of heartworms through increase in total pulmonary resistance. Decrease in venous

return might be followed by decrease in circulating blood volume or delatation of peripheral vessels, which could induce by infusion of body fluid extracts of heartworms. Moreover, decrease in cardiac output following to diminished pumping ability of the heart was brought about by the valvular and muscular heart diseases, heart failure, and depression by dominant nerves [39]. In dogs with CS, heartworm migration may be induced with one or more complex combinations of these factors to decrease cardiac output. However, death of heartworms may be a primary cause of heartworm migration from the pulmonary arteries towards the right atrium, because thromboemboli including recently died heartworms were found in the pulmonary arteries of almost all dogs with naturally acquired CS. As shown in this study, the death of heartworms reduces right cardiac output through the shock-like reaction pulmonary thromboembolism and both.

6. SUMMARY

Caval syndrome develops in a small part of dogs with dirofilariasis. Violent symptoms such as prostration, hemoglobinuria, anemia, dyspnea, and cardiac murmur develop suddenly. Because most dogs affected die with acute course, CS has been regarded as the important diseases in veterinary clinical medicine. It has been considered that heartworms located at the venae cavae might play an important role in pathophysiology of the disease. However, there were some cases in which heartworms could not be found at the venae cavae, and symptoms could not be induced by insertion of heartworm-like electric wire into the venae cavae of the dog. Thus, the theory has not been established in location of heartworms associated closely with pathophysiology of CS. Some clinicopathological studies have been carried out in dogs with naturally acquired CS. However, hemodynamics have not been determined, in spite of severe symptoms of circulatory disturbance. Also, relationship between the location of heartworms and circulatory disturbance has been unknown. Besides, many dogs with CS excrete hemoglobinuria indicating increase in intravascular hemolysis. The mechanism of intravascular hemolysis has also unknown. In SECTION I, the present study attempted to make clear the location of heartworms associated closely with circulatory disturbance in CS. Cardiopulmonary function values were measured before and after surgical heartworm removal in dogs with naturally acquired CS, and were measured in dogs with artificial model

of CS produced by insertion of heartworm-like silicone tubes into the tricuspid valve orifice. In SECTION II, the author probed mechanism of intravascular hemolysis, which is one of the characteristic symptoms in CS. Relationship between the location of heartworms and plasma hemoglobin concentration, and other possible factors inducing intravascular hemolysis were examined.

The onset of CS is migration of heartworms from the pulmonary arteries to the tricuspid valve orifice. The cause of heartworm migration has not been investigated precisely. It was only suggested that the parasitism of a large number (overcrowding) of heartworms in the pulmonary arteries might induce heartworm migration from the pulmonary arteries towards the right atrium. In SECTION III, the study investigated the factors inducing heartworm migration from the pulmonary arteries. The author attempted to induce heartworm migration to make change hemodynamics of dogs in which heartworms resided only in the pulmonary arteries. Results are summaried as follows.

I. Parasitic location of heartworms and right heart hemodynamics

On echocardiographic findings and post-mortem examinations, heartworms located at the tricuspid valve orifice between the right atrium and right ventricle in all dogs with naturally acquired CS. On angiocardiography, a contrast medium regurgitated from the right ventricle to the

right atrium, and pulmonary arterial embolization were observed in most dogs with CS. Central venous pressure (right atrial pressure) was high, and indicated severe dysfunction of the tricuspid valve such as stenosis and regurgitation. After surgical heartworm removal from the tricuspid valve orifice, clinical signs were improved in many dogs, and central venous pressure (right atrial pressure) decreased, but pulmonary arterial pressure did not alter obviously. Right cardiac output increased significantly in dogs relieved after heartworm removal, but did not increased in dogs having ominous prognosis. By insertion of heartworm-like silicone tubes into the tricuspid valve orifice, symptoms such as decreases in vigor and appetite, anemia, systolic cardiac murmur, jugular pulsation, dyspnea, hemoglobinuria, and ascites were reproduced. After tube insertion, a contrast medium regurgitated to the right atrium, and both a- and v-wave of right atrial pressure elevated. Pulmonary arterial pressure did not elevate obviously, but right cardiac output decreased. Changes in right heart hemodynamics were greater in dogs inserted a large number of tubes than in dogs inserted a small number of tubes. From these findings, it is concludeed that heartworms at the tricuspid valve orifice bring about tricuspid valve dysfunction, and circulatory disturbance caused by tricuspid valve dysfunction and pulmonary thromboembolization may relate closely with the development of clinical signs of CS.

II. Mechanism of intravascular hemolysis

Immediately after surgical removal of heartworms at the tricuspid valve orifice, plasma hemoglobin concentration decreased acutely. In contrast, plasma hemoglobin concentration increased immediately after migration of heartworm at the tricuspid valve orifice in dogs developed CS after administration of milbemycin D. In dogs inserted a large number of silicone tubes at the right atrium and tricuspid valve orifice, increase in plasma hemoglobin concentration was greater than in dogs inserted a small number of tubes. In peripheral blood films of dogs with naturally acquired CS, fragmented erythrocytes such as scizocytes and microspherocytes, which suggested mechanical hemolysis, were observed as well as codocytes and acanthocytes, in which components of erythrocyte membrane increased. Erythrocyte osmotic fragility (50 % hemolysis) was normal, but mechanical fragility increased in dogs with CS. Liver dysfunction resulted from severe circulatory disturbance induced low plasma activity of lecithin: cholesterol acyltransferase, then free cholesterol content increased in low density lipoprotein and erythrocyte membranes. Erythrocyte mechanical fragility increased because of increase in free cholesterol content in erythrocyte membrane. On the other hand, serum bile acid concentration, plasma osmolality, blood ATP level, and serum and erythrocyte-membrane phospholipids did not associate with intravascular hemolysis. Direct Coombs' test was negative, and disseminated intravascular coagulation was also denied from

results of blood coagulation and fiblinolysis examinations and histopathological examination. These findings indicated that immunological and chemical mechanisms did not relate with intravascular hemolysis. In this disease, heartworms at the tricuspid valve orifice induce abnormal bloodstream flow. Under conditions of abnormal bloodstream flow, it is considered that erythrocytes having a predisposing factor to be injured easily will be readily destroyed by the shearing force and physical collision to the cardiac wall or surface of heartworms.

III. Factors inducing migration of heartworms from pulmonary arteries towards right atrium

In heartworm-infected dogs administered milbemycin D, a prophylactic for heartworm infection, live heartworms migrated from the pulmonary arteries towards the right atrium. Filarid-worm echoes like as natural cases were observed at tricuspid valve orifice by echocardiography. In dogs with heartworm migration, right cardiac output decreased markedly. Then, in order to diminish right cardiac output, a $beta_1$-blocker (metoprolol) administered to heartworm-infected dogs. Heartworms also migrated to the tricuspid valve orifice after treatment. At the time of heartworm migration, changes in pulmonary arterial pressure were not uniform, but heart rate and right cardiac output decreased obviously. Nextly, in order to investigate relationship between the heartworm migration and death of heartworms, dead heartworms were inserted into

the pulmonary arteries of 14 heartworm-infected dogs. Live heartworms migrated to the tricuspid valve orifice in 6 dogs immediately after insertion of dead worms, and live worms migrated in 4 dogs 1 to 7 days after insertion. In dogs with immediately heartworm migration, pulmonary arterial pressure fell, and right cardiac output decreased to unmeasurably low level (<1.0 L/min). In dogs with heartworm migration 1 to 7 days after insertion of death worms, pulmonary arterial pressure elevated, and right cardiac output decreased gradually. Because it was considered that changes in hemodynamics might associate with substances including in body fluid released from dead heartworms, a body-fluid extract from adult female heartworms was infused intravenously. Heartworms migrated within 15 minutes after body-fluid infusion, and pulmonary arterial pressure fell, and right cardiac output decreased markedly. Systemic blood pressure also fell, and suggested the occurrence of shock-like reaction. Besides, in order to reproduce pulmonary thromboembolization, heartworm-like silicone tubes were inserted into the pulmonary arteries of dogs with heartworm infection. Live heartworms migrated in 4 of 11 dogs 1 to 7 days after insertion of tubes. Pulmonary arterial pressure elevated gradually, and right cardiac output decreased. It was concluded that heartworm migration from the pulmonary arteries towards the right atrium associated with decrease in right cardiac output. Moreover, in dogs with naturally acquired CS, death of adult heartworms may induce migration of other live heartworms through the decrease in

right cardiac output resulted from shock-like reaction or increase in pulmonary vascular resistance.

Pathophysiology of CS was investigated from parasitic location of heartworms, right heart hemodynamics and mechanism of intravascular hemolysis. Results indicated that the occurrence of the disease related closely with migration of heartworms from the pulmonary arteries and presence of heartworms at the tricuspid valve orifice. Because heartworm migration was noticed when right cardiac output decreased following infusion of heartworm-body fluid and pulmonary thromboembolization, it was considered that the heartworm migration to the tricuspid valve orifice might result from death of a part of heartworms residing. Tricuspid valve function was interfered with heartworms at there. Thus, circulatory disturbance induced by tricuspid valve dysfunction and pulmonary thromboembolization might associate with development of symptoms in CS. Heartworms at the tricuspid valve orifice also induced abnormal bloodstream flow. Circulating erythrocytes, which have acquired the tendency to be injured easily resulted from liver dysfunction, might be destroyed by the physical mechanism.

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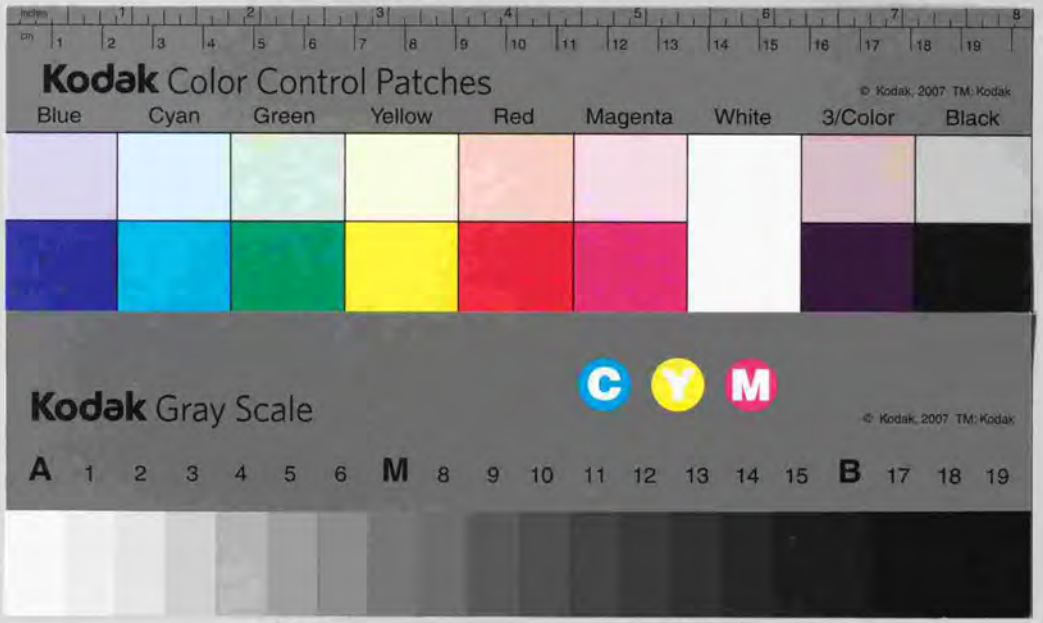
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