Lipids in Serum, Lipoproteins and Erythrocyte Membranes in Canine Dirofilariasis by TLC-FID Method

Hitoshi KITAGAWA, Yoshihide SASAKI, Masaaki MORI, and Katsuya ISHIHARA

Department of Veterinary Internal Medicine, Faculty of Agriculture, Gifu University, 1-1 Yanagido, Gifu 501-11, Japan

(Received 26 May 1986/Accepted 23 December 1986)

ABSTRACT. The lipids in serum, lipoproteins and erythrocyte membranes were analyzed by the thin-layer chromatography-flame ionization detector (TLC-FID) method in normal dogs (control group, n=18) and dogs with dirofilarial hemoglobinuria (hemoglobinuria group, n=19) and chronic serious dirofilariasis (serious group, n=12). Compared with the control group, the hemoglobinuria and serious groups showed a higher serum free cholesterol (FC) level and erythrocyte membrane FC content, and lower serum cholesterol ester (CE) ratio. The serum triglyceride (TG) level in the serious group was higher. Phospholipid (PL) profile in the hemoglobinuria group revealed that serum and erythrocyte membrane sphingomyelin (SM) levels were higher, but other PL levels were not higher. In the serious group, the serum lysophosphatidyl choline (lyso-PC) level was lower, whereas the serum SM, erythrocyte membrane phosphatidyl serine and total lipid levels were higher. The hemoglobinuria group had a low level of high density lipoprotein (HDL), high levels of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in serum. CE ratio and lyso-PC content in HDL were lower, and all lipid contents in LDL in the hemoglobinuria group were higher. The increases of FC contents in serum and erythrocyte membrane related to the increase of FC content in LDL, and the decreases of PL contents other than SM related to the decrease of serum HDL concentration. Though the difference from the control group was greater, the lipid metabolism in the hemoglobinuria group might be fundamentally similar to that in the serious group. —**KEY WORDS:** dirofilariasis, dog, lipid, TLC-FID.

Jpn. J. Vet. Sci. 49(2): 285–293, 1987

Plasma or tissue lipids have been quantitatively analyzed by chemical methods or thin-layer chromatography. Recently, a thin-layer chromatography-flame ionization detector (TLC-FID) system has been developed and applied to the analysis of lipids [1, 13, 22, 24, 25]. In this method, TLC is performed on a "Chromarod," a quartz rod with silica gel coating, and each separated lipid is analyzed neutral lipids and phospholipids, and has both high sensitivity and good reproducibility [1, 13]. Previously, the authors reported on the serum and erythrocyte membrane lipid profile in dogs with dirofilariasis by the chemical method [7, 8]. However, the findings of the triglyceride, phospholipid fractions and serum lipoprotein lipid contents were not mentioned in these studies. In the present study, the lipids

in serum, serum lipoproteins and erythrocyte membranes were analyzed in normal dogs and dogs with chronic serious dirofilariasis and dirofilarial hemoglobinuria by the TLC-FID method. In hemoglobinuria cases, in order to examine the relationship between the lipid profile and intravascular hemolysis, lipid profile was analyzed also 20 hr after surgical heartworm removal.

MATERIALS AND METHODS

Dogs: As the normal control, 13 normal and parasite-free beagles and 5 healthy Japanese mongrels, consisting of 8 males and 10 non-pregnant bitches, were used (control group). The age of the control dogs was 2 to 5 years old. The beagles were bred in C.S.K. Laboratory Animals Co., Ltd.

(Minowa-cho, Nagano, Japan) with a commercial dog food, and blood samples only were supplied from the laboratory. The mongrel dogs were obtained from the regional health center, and fed on a commercial dog food in our laboratory. These mongrel dogs were confirmed to be dirofilariasis-free by autopsy. Thirty-one dogs with dirofilariasis were used. They were divided into 2 groups from their clinical signs and laboratory test results. The serious group included 12 dogs with the signs of chronic serious dirofilariasis such as severe cardiac insufficiency, respiratory abnormality, ascite and/or jaundice. This group consisted of 8 males and 4 females aged from 5 to 10 years old. The hemoglobinuria group included 19 dogs with dirofilarial hemoglobinuria, and 15 males and 4 females aged from 2 to 10 years old. These dogs displayed the signs of so-called "caval syndrome" [6, 11]. Eleven dogs underwent surgical heartworm removal from the jugular vein [3], and all recovered. The clinical signs and laboratory test results in the control, serious and hemoglobinuria groups were almost similar to those in our previous reports [6, 7, 15].

Methods: Peripheral blood samples were collected from the fasting animals. Blood serum was separated as soon as possible and analyzed on the same day. Serum lipoproteins were fractionated by cellulose acetate electrophoresis, and the concentration of each fraction was calculated according to the method of Ishihara *et al.* [9]. To analyze the lipid contents, serum lipoproteins were separated by ultracentrifugation with reference to the method of Havel *et al.* [5], and Ishihara *et al.* [9]. Serum and lipoprotein lipids were extracted from 0.5 ml of samples by the Folch method [2] using chloroform-methanol (2:1, v/v). Erythrocytes were washed 5 or 6 times by 0.85% saline. Washed erythrocytes (0.9 ml) were hemolyzed by 1.0 ml of distilled water, and lipids were extracted by the Rose method [21]

using 11 ml of iso-propanol and 7 ml of chloroform. Lipid extracts were applied to the Chromarod S-II (Iatron Labs. Inc. Tokyo). The Chromarods were developed 2 times in chloroform-methanol-water (40:20:2.5, v/v) for the separation of serum phospholipids, and re-developed in n-hexan-diethyl ether-formic acid (54:6:0.08, v/v) for neutral lipids. For separation of erythrocyte membrane lipids, the Chromarods were developed in the former mobile phase. After drying, the Chromarods were scanned through a flame ionization detector (Iatroscan TH-10 TLC-FLD Analyser, Iatron Labs. Inc., Tokyo). The best 3 of 5 chromatograms were selected for 1 sample, and their mean area was used to calculate the concentration. Serum lipid concentrations were calculated from the area ratio for an internal standard substance (cholesterol acetate). Erythrocyte membrane lipid contents were calculated from the area ratio for the free cholesterol content, which was determined by the enzymatic method (Iatro-lipo-TC Kit, Iatron Labs. Inc., Tokyo). For comparison of the data, when the variances seemed to be uniform (Bartlett's test for uniformity of variance), Duncan's new multiple range test was used. When not uniform, Student's t-test or Cochran-Cox test was used.

RESULTS

Fig. 1 shows a lipid chromatogram of serum, and Table 1 indicates the mean concentrations of serum lipids in 3 groups. In the serious group, the cholesterol ester (CE) level was almost similar, but the free cholesterol (FC) level tended to be higher, making for a significantly lower CE ratio than in the control group. Triglyceride (TG) level was higher than in the control group. Phosphatidyl choline (PC) level was slightly but not significantly lower, sphingomyelin (SM) level significantly higher, and

Fig. 1. Lipid chromatogram of serum in a control dog. 1: Cholesterol ester, 2: Cholesterol acetate, 3: Triglyceride, 4: Free fatty acid, 5: Free cholesterol, 6: Phosphatidyl choline, 7: Sphingomyelin, 8: Lysophosphatidyl choline.

lysophosphatidyl choline (lyso-PC) level lower, respectively, than in the control group. In the hemoglobinuria group, compared with the control group, FC and SM levels were higher, and CE, CE ratio, PC, lyso-PC, total phospholipid (TPL) and total lipid (TL) levels lower.

Serum lipoprotein was analyzed in the control and hemoglobinuria groups (Table 2). In the hemoglobinuria group, α -lipoprotein (high density lipoprotein, HDL) level was lower, but β - (low density lipoprotein, LDL) and pre- β - (very low density lipoprotein, VLDL) lipoproteins were higher than in the control group.

Table 3 shows the lipid contents in each lipoprotein fraction in the control and hemoglobinuria groups. In the HDL frac-

Fig. 2. Lipid chromatogram of erythrocyte membranes in a control dog. 1: Free cholesterol, 2: Phosphatidyl ethanolamine, 3: Phosphatidyl serine, 4: Phosphatidyl choline, 5: Sphingomyelin.

tion, the hemoglobinuria group had lower levels of CE ratio, and lyso-PC. In the LDL fraction, the hemoglobinuria group had high levels of CE, FC, TG, PC, SM, lyso-PC, TPL and TL. In the VLDL fraction, there were no statistically significant differences in the lipid contents between the control and hemoglobinuria groups. FC contents in serum and erythrocyte membrane correlated significantly to FC content in LDL, and PL contents other than SM correlated to serum HDL concentration.

Fig. 2 shows a chromatogram of erythrocyte membrane lipids, and Table 4 shows

Table 1. Serum lipid concentrations in 3 groups

Item	Control			Serious			Hemoglobinuria			p< ^{b)}
	n	Mean	SD ^{a)}	n	Mean	SD	n	Mean	SD	
Cholesterol ester (mg/dl)	18	241.2	64.0	12	229.4	102.7	17	155.3	84.8**	0.01
Free cholesterol (mg/dl)	18	53.4	8.6	12	83.1	46.7	17	77.6	27.9**	NS
Cholesterol ester ratio (%)	18	81.3	3.3	12	73.5	6.0**d)	17	65.8	11.1**	0.05
Triglyceride (mg/dl)	17	25.3	13.6	12	71.4	60.9**e)	17	36.7	21.1	NS
Phosphatidyl choline (mg/dl)	18	348.4	83.1	12	287.2	110.2	17	182.5	120.3**	0.01
Sphingomyelin (mg/dl)	18	29.1	6.4	12	35.6	10.8**	17	43.8	17.5**	0.01
Lysophosphatidyl choline (mg/dl)	18	25.1	7.1	12	17.7	5.8**	17	16.9	8.5**	NS
Total phospholipid (mg/dl)	18	402.5	92.2	12	355.0	143.3	17	243.5	127.7**	0.01
Total lipid (mg/dl)	17	718.7	160.4	12	738.9	326.0	17	512.8	233.9**	NS

a) Standard deviation.

b) Probability of significant difference between the serious and hemoglobinuria groups.

c) Not significant.

d, e) Probability of significant difference for the control group at p<0.01 (d) and p<0.05 (e), respectively.

Table 3. Lipoprotein lipid contents in the control and hemoglobinuria groups

Item	High density lipoprotein						p< ^(b)
	Control			Hemoglobinuria			
	n	Mean	SD ^{a)}	n	Mean	SD	
Cholesterol ester (mg/dl ^{c)})	7	245.8	31.2	4	150.2	120.9	NS ^{d)}
Free cholesterol (mg/dl)	7	40.1	3.5	4	40.5	27.5	NS
Cholesterol ester ratio (%)	7	85.9	1.2	4	77.8	3.3	0.05
Triglyceride (mg/dl)	7	2.0	1.5	4	3.2	1.3	NS
Phosphatidyl choline (mg/dl)	7	341.8	29.0	4	225.1	103.4	NS
Sphingomyelin (mg/dl)	7	23.3	2.7	4	24.4	20.9	NS
Lysophosphatidyl choline (mg/dl)	7	27.9	4.8	4	13.2	5.0	0.01
Total phospholipid (mg/dl)	7	393.0	32.9	4	262.7	128.1	NS
Total lipid (mg/dl)	7	680.9	57.6	4	456.6	274.8	NS

a) Standard deviation.

b) Probability of significant difference.

c) mg/dl serum.

d) Not significant.

the erythrocyte membrane lipid contents in 3 groups. The serious group had higher levels of FC, phosphatidyl serine (PS), TPL and TL than the control group. FC/PL ratio was slightly higher, but not statistically significant, because both FC and PL were higher. The hemoglobinuria group had higher FC, SM, TL and FC/PL ratio than the control group. The contents of phosphatidyl ethanolamine (PE), PS and PC were

almost the same as in the control group.

Table 5 shows the lipid profiles before and 20 hr after surgical heartworm removal in the hemoglobinuria group. After heartworm removal, serum TG was significantly higher and SM lower. Erythrocyte membrane FC, PE, PC SM, TPL and TL were higher. The lipid profile 20 hr after heartworm removal tended to be more similar to that in the serious group, that is, higher TG

Table 2. Serum lipoprotein concentrations in the control and hemoglobinuria groups

Item		Control			Hemoglobinuria			p< ^{b)}
		n	Mean	SD ^{a)}	n	Mean	SD	
α -lipoprotein	(mg/dl)	13	1221.3	126.6	6	567.0	126.4	0.01
	(%)		86.4	5.8		43.0	10.4	0.01
β -lipoprotein	(mg/dl)	13	54.2	29.9	6	269.1	95.2	0.01
	(%)		4.7	2.6		24.2	7.8	0.01
Pre- β -lipoprotein	(mg/dl)	13	81.4	45.6	6	302.1	166.0	0.05
	(%)		8.9	4.7		32.9	11.8	0.01

a) Standard deviation.

b) Probability of significant difference.

Low density lipoprotein							Very low density lipoprotein						
Control			Hemoglobinuria			p<	Control			Hemoglobinuria			p<
n	Mean	SD	n	Mean	SD		n	Mean	SD	n	Mean	SD	
7	13.3	6.9	4	59.8	23.3	0.05	7	14.9	6.7	4	20.9	26.9	NS
7	6.5	2.2	4	31.6	4.5	0.01	7	9.1	3.0	4	16.9	12.9	NS
7	65.9	6.5	4	63.7	7.9	NS	7	60.5	9.4	4	45.2	14.0	NS
7	3.0	1.4	4	21.0	5.7	0.01	7	17.9	1.5	4	29.0	10.6	NS
7	25.1	12.9	4	83.5	33.6	0.05	7	39.9	19.1	4	40.2	34.9	NS
7	3.0	2.9	4	13.6	6.8	0.05	7	4.6	0.7	4	7.1	5.5	NS
7	0	0	4	5.3	2.9	0.01	7	2.5	1.7	4	1.0	1.5	NS
7	28.1	15.3	4	102.3	35.0	0.01	7	46.9	20.9	4	48.1	41.6	NS
7	51.0	23.1	4	214.7	56.1	0.01	7	83.4	28.0	4	114.9	90.0	NS

and lower SM levels in serum and higher tendency of FC and each PL in erythrocyte membranes.

DISCUSSION

The control data in the present study were compared with those in our previous studies [6–9] and those of others [14, 18], in which the lipid analysis was performed by chemical

methods. In the present study, the serum lipoprotein concentrations in the present study were higher, because serum total lipid concentration measured by the TLC-FID method was higher than by the sulfo-phospho-vanillin method (unpublished data). The serum CE and TPL levels were considerably higher, while the FC level was slightly higher. On the contrary, the serum TG level was lower. The CE ratio was

Table 4. Erythrocyte membrane lipid contents in 3 groups

Item	Control			Serious			Hemoglobinuria			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 ^{**d)}	18	7.48	1.35 ^{**}	NS ^{c)}
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	1.09	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 ^{*e)}	18	20.32	3.37 [*]	NS
Free 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 hemoglobinuria groups.

c) Not significant.

d, e) Probability of significant difference for the control group; each asterisk indicates at p<0.01 (d) and p<0.05 (e), respectively.

Table 5. Serum and erythrocyte membrane lipid levels before and 20 hr after heartworm removal in the hemoglobinuria group

Item	n	Before removal		20 hr after removal		p< ^{a)}
		Mean	SD ^{b)}	Mean	SD	
Serum lipid						
Cholesterol ester (mg/dl)	11	188.9	87.5	194.1	96.6	NS ^{c)}
Free cholesterol (mg/dl)	11	82.6	22.4	83.4	35.6	NS
Cholesterol ester ratio (%)	11	69.2	8.3	69.2	4.1	NS
Triglyceride (mg/dl)	11	38.5	22.3	63.1	45.1	0.05
Phosphatidyl choline (mg/dl)	11	222.5	129.7	265.1	118.4	NS
Sphingomyelin (mg/dl)	11	49.2	17.7	32.5	15.9	0.01
Lysophosphatidyl choline (mg/dl)	11	20.6	7.1	16.0	6.2	NS
Total phospholipid (mg/dl)	11	292.2	130.7	313.6	121.5	NS
Total lipid (mg/dl)	11	602.3	239.5	654.2	268.5	NS
Erythrocyte membrane lipid						
Free cholesterol (mg/gHb)	11	6.79	0.84	7.76	1.18	0.025
Phosphatidyl ethanolamine (mg/gHb)	11	3.11	1.13	3.99	1.63	0.01
Phosphatidyl serine (mg/gHb)	11	1.58	0.72	1.81	0.63	NS
Phosphatidyl choline (mg/gHb)	11	7.40	1.50	8.55	1.97	0.05
Sphingomyelin (mg/gHb)	11	0.89	0.32	1.34	0.58	0.025
Total phospholipid (mg/gHb)	11	12.98	2.91	15.69	4.05	0.025
Total lipid (mg/gHb)	11	19.77	3.57	23.45	4.94	0.025
Free cholesterol/phospholipid ratio	11	0.54	0.12	0.52	0.15	NS

a) Probability of significant difference.

b) Standard deviation.

c) Not significant.

rather similar to our previous findings [7, 8], and the PC level approached that in Mahleys' data [18]. The erythrocyte mem-

brane FC and TPL and FC/PL ratio were virtually the same as in our previous study [7]. The lipid profile was influenced by

many factors such as breed, age, sex, season, feed, life environment and so on. The nutrition of the control dogs was relatively good and possibly one reason for the high lipid levels in the control group of the present study. The difference of analytical procedure might also serve to explain the differences in the results. However, the data in the control dogs may be of value as the basic control data for the findings in the diseased dogs to be analysed by the TLC-FLD method. When the data in diseased dogs were compared with that in control dogs, the differences of breed, age, sex and so on had to be taken into consideration. However, the changes in lipid profile associated with the dirofilariasis might be greater than those associated with breed, age, sex and so on.

In the hemoglobinuria group, there were increases of serum LDL concentration and serum FC concentration, and a decrease in the CE ratio. These findings corroborated our previous findings [8, 10]. The increase in serum FC concentration was the result of the increase in FC content in LDL. The authors already reported that low serum lecithin cholesterol acyltransferase (LCAT) activity was one cause of the high serum FC level in the hemoglobinuria and serious groups. Moreover, increase of serum LDL concentration suggested a decrease in LDL uptake from plasma by cells of the liver and other organs. These might have resulted from saturation of cholesterol in cells and/or the suppression of LDL receptors. The high erythrocyte FC content also should be brought about by increased FC content in serum LDL.

In the PL fractions, the hemoglobinuria group had low PC and lyso-PC concentrations in serum. These findings were the same as in human hepatic cirrhosis [4]. The decreases of these PL were related to the decrease of serum HDL concentration. These findings indicated the decreases of

HDL production by the liver and plasma LCAT activity. Glycerophospholipid (PE, PS and PC) contents in erythrocyte membranes were not significantly different among 3 groups except PS in the serious group. SM contents in serum and erythrocyte membranes were higher in the hemoglobinuria group. The SM metabolism is closely associated with the cholesterol metabolism. The SM uptake by cells was suppressed by the increased cholesterol content in cells [19]. The increases of cholesterol in serum and erythrocyte membranes might suggest the decreased uptake of SM by cells from plasma. The PL contents except for SM in serum and erythrocyte membrane were normal or low in the hemoglobinuria group. There was no report on the intravascular hemolysis caused by SM. Though the PL, especially lyso-PC, had a hemolytic effect [26], the results in the present study indicated that the accelerated intravascular hemolysis did not involve PL in the hemoglobinuria group, as in familial plasma LCAT deficiency [20] or high PC hemolytic anemia [12] in humans. The easily injured disposition of erythrocytes was again suggested from the higher erythrocyte membrane FC content and FC/PL ratio in the hemoglobinuria group. However, only the abnormal lipid profile in erythrocyte membranes did not bring about the accelerated intravascular hemolysis. The severe intravascular hemolysis may have resulted from abnormal bloodstream flow by heartworm mass at the tricuspid valve orifice [7, 16] and tendency in erythrocytes to be injured easily [6, 7] in the hemoglobinuria group.

The lipid profile in the hemoglobinuria group showed a fundamentally similar tendency to that in the serious group. The lipid profile 20 hr after heartworm removal was also relatively similar to that in the serious group. Moreover, the early stage of dirofilarial hemoglobinuria, which developed after administration of milbemycin D, a macro-

lide anthelmintic [23], showed a lipid profile like those in the serious group [17]. The abnormality of the lipid metabolism in the hemoglobinuria group might be fundamentally similar to that in the serious group.

REFERENCES

- Bradley, D. M., Rickards, C. R., and Thomas, N. S. T. 1979. Plasma lipid analysis by thin-layer chromatography with flame ionization detection and quantitation. *Clin. Chim. Acta* 92: 293-302.
- Folch, J., Lees, M., and Sloane-Stanley, G. H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226: 497-509.
- Fujii, I. 1975. A clinical study on the venae cavae embolism by heartworms of dogs. *Bull. Azabu Vet. Coll.* No. 30: 105-118 (in Japanese).
- Gjone, E., and Orning, O. M. 1966. Plasma phospholipids in patients with liver disease. A quantitative thin layer chromatographic study. *Scand. J. Clin. Invest.* 18: 209-216.
- Havel, R. J., Eder, H. A., and Bragdon, J. H. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34: 1344-1353.
- Ishihara, K., Kitagawa, H., Ojima, M., Yagata, Y., and Suganuma, Y. 1978. Clinicopathological studies on canine dirofilariasis hemoglobinuria. *Jpn. J. Vet. Sci.* 40: 525-537.
- Ishihara, K., Kitagawa, K., Yokoyama, S., Ohashi, H. 1981. Studies on hemolysis in canine dirofilariasis hemoglobinuria. Lipid alterations in blood serum and red cell membrane. *Jpn. J. Vet. Sci.* 43: 1-11.
- Ishihara, K., Ojima, M., Kitagawa, H., and Suganuma, Y. 1978. Studies on serum free cholesterol concentration and serum lecithin cholesterol acyltransferase activity in canine dirofilariasis. *Jpn. J. Vet. Sci.* 40: 171-183.
- Ishihara, K., Suganuma, Y., and Watanabe, Y. 1977. Electrophoretic studies on serum lipoprotein fractions in normal dogs. *Jpn. J. Vet. Sci.* 39: 149-157.
- Ishihara, K., Suganuma, Y., Watanabe, Y., Ojima, M., and Kitagawa, H. 1977. Electrophoretic studies on serum lipoprotein fractions in canine dirofilariasis. *Jpn. J. Vet. Sci.* 39: 255-264.
- Jackson, R. F., Seymour, W. G., Grownay, P. J., and Otto, G. F. 1977. Surgical treatment of the caval syndrome of canine heartworm disease. *J. Am. Vet. Med. Assoc.* 171: 1065-1069.
- Jaffe, E. R., and Gottfried, E. L. 1968. Hereditary non-spherocytic hemolytic disease associated with an altered phospholipid composition of the erythrocytes. *J. Clin. Invest.* 47: 1375-1388.
- Kaitaranta, J. K., and Nicolaides, N. 1981. Response and linearity of different lipid compounds when analyzed by thin-layer chromatography with flame ionization detection. *J. Chromat.* 205: 339-347.
- Kaneko, J. J. 1980. Normal concentrations of blood constituents in domestic animals. pp. 792-795. In: *Clinical Biochemistry of Domestic Animals*. (Kaneko, J. J. ed.). Academic Press, New York.
- Kitagawa, H., Ishihara, K., Yokoyama, S., and Sasaki, Y. 1982. Serum total bile acid levels in canine dirofilariasis. *Jpn. J. Vet. Sci.* 44: 917-923.
- Kitagawa, H., Sasaki, Y., and Ishihara, K. 1986. Clinical studies on canine dirofilariasis hemoglobinuria: Relationship between the presence of heartworm mass at the tricuspid valve orifice and plasma hemoglobin concentration. *Jpn. J. Vet. Sci.* 48: 99-103.
- Kitagawa, H., Sasaki, Y., and Ishihara, K. 1986. Canine dirofilariasis hemoglobinuria induced by milbemycin D administration. *Jpn. J. Vet. Sci.* 48: 517-522.
- Mahley, R. W., and Weisgraber, K. H. 1974. Canine lipoproteins and atherosclerosis. I. Isolation and characterization of plasma lipoproteins from control dogs. *Circ. Res.* 35: 713-721.
- Mazière, J. C., Mazière, C., Mora, L., and Polonovski, J. 1984. Impairment of exogenous sphingomyelin degradation in cultured fibroblasts from familial hypercholesterolemia. *FEBS Lett.* 173: 159-163.
- Norum, K. R., and Gjone, E. 1967. Familial plasma lecithin: cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab. Invest.* 20: 231-243.
- Rose, H. G., and Oklander, M. 1965. Improved procedure for the extraction of lipids from human erythrocytes. *J. Lipid Res.* 6: 428-431.
- Szaskits, J. J., Peurifoy, P. V., and Woods, L. A. 1970. Quantitative thin-layer chromatography using a flame ionization detector. *Anal. Chem.* 42: 351-354.
- Takiguchi, Y., Mishima, H., Okuda, M., and Terao, M. 1980. Milbemycins, a new family of macrolide antibiotics: Fermentation, isolation and physico-chemical properties. *J. Antibiotics* 33: 1120-1127.
- Tanaka, M., Itoh, T., and Kaneko, H. 1976. Quantitative determination of neutral lipids on thin layer-FID chromatography. *J. Jpn. Oil Chem. Soc.* 25: 263-265 (in Japanese).
- Tanaka, M., Itoh, T., and Kaneko, H. 1977. Quantitative determination of polar lipids on thin layer-FID chromatography. *J. Jpn. Oil Chem.*

- Soc.* 26: 454-457 (in Japanese).
26. Ueda, N. 1983. The chemistry of plasma lipoprotein. 3. 1. Lipids. *In*: Plasma Lipoprotein. pp. 21-30. (Hara, I., Ueda, N., and Akanuma, Y. eds.). Kodansha, Tokyo (in Japanese).

要 約

TLC-FID 法による犬糸状虫症における血清, リポ蛋白質及び赤血球膜の脂質所見: 北川 均・佐々木栄英・森 昌昭・石原勝也 (岐阜大学農学部家畜内科学講座)——健康犬(対照群, n=18), 犬糸状虫性血色素尿症(血色素尿症群, n=19)及び慢性重症の犬糸状虫症(重症群, n=12)の血清, リポ蛋白質及び赤血球膜の脂質を TLC-FID (薄層クロマトグラフィー・水素炎イオン化検出法) 法により分析定量した。対照群と比較して, 血色素尿症群と重症群では, 血清 free cholesterol (FC) 濃度, 赤血球膜 FC 含量及び FC/PL 比は高く, 血清 cholesterol ester (CE) 比は低値で, 既報の化学的方法による成績とほぼ一致した。血清 triglyceride (TG) 濃度は重症群で高値であった。血色素尿症群では, 血清と赤血球膜の sphingomyelin (SM) は高値であったが, 他の PL は高値を示さず, この群にみる血管内溶血は PL との直接的関連性を指摘できなかった。慢性重症群では, 血清の lysophosphatidyl choline (lyso-PC) 濃度は低く, 血清の SM, 赤血球膜の phosphatidyl serine, 及び総 PL 量は高値であった。血色素尿症群の高比重リポ蛋白質 (HDL) 濃度は低く, 低比重 (LDL) 及び超低比重 (VLDL) リポ蛋白質濃度は高かった。また, HDL の CE, PC, lyso-PC 及び総 PL 量は低く, LDL では FC をはじめすべての脂質分画が高値であった。血清と赤血球膜の FC の増加は LDL の FC 増加が原因であり, SM 以外の PL の減少は血清 HDL 濃度の減少と関連していた。血色素尿症群の脂質代謝異常はさらに顕著であったが, 重症群類似の傾向と考えられた。