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CYTOLOGICAL STUDIES ON SIDEROCYTES IN EQUINE INFECTIOUS ANEMIA

II. SYSTEMATIC OBSERVATIONS OF SIDEROCYTES

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PREFACE

The author carried out cytological studies of siderocytes in the blood of jugular vein of horses affected and non affected with EIA (equine infectious anemia) in the previous report. It was recognized that siderocytes be classified cytologically into five varieties which consist of siderophagous neutrophilic leucocyte, siderophagous monocyte, large siderophagocyte, small siderophagocyte and small siderophagous lymphoid cell. It was also found that the appearance of siderocytes is closely related with the histological findings. For the cytogenesis of those cells, it was concluded: siderophagous neutrophilic leucocytes and siderophagous monocytes are the native blood cells; the large siderophagocyte is a reticulo-endothelial cell in narrow sense; the small siderophagocyte originates in AKAZAKI's type II phagocyte^{1,13,14,17,18}; the small siderophagous lymphoid cell originates in small lymphoid cell. Experimental observation of siderocytes in the blood of jugular vein of the horse affected with EIA were carried out by ISHII (1939, 1940)^{6,7} and ISHII et al. (1940)¹¹. In his compendious explanation, ISHII emphasized that the appearance of siderocytes is recognized at the stage of histiocytic reaction because siderocytes originate in histiocytic cells. His opinion does not always coincide with the author's result. Therefore, the author attempted, for the amplification of his findings, some systematic investigations of siderocytes in experimental cases.

MATERIALS AND METHODS

The fluctuation in the ratio of appearance of siderocytes and the proportion of cytological varieties of siderocytes in the blood of the jugular vein were observed in three artificially affected cases (all chronic type EIA) and two naturally affected cases (each one case of chronic and relapsed type EIA) for a long period. Siderocytes in the splanchnic blood vessel of the three cases of chronic type EIA and one case of relapsed type EIA were observed systematically. These horses were subjected to laparotomy soon after the amputation of the medulla oblongata, and blood samples were obtained from the anterior mesenteric vein, splenic vein, portal vein, hepatic vein, posterior vena cava, anterior vena

cava, pulmonary artery, pulmonary vein, common carotid artery and jugular vein. In one case each of chronic and relapsed type EIA, lymph was obtained from the thoracic duct and the ascites was obtained from the abdominal cavity. In that condition, a part of the above mentioned humor was smeared immediately over the slides, and they were investigated use being made of MAY-GIEMSA staining and ARMITAG's peroxydase reaction. Their hemograms were counted in MAY-GIEMSA stained preparation of the blood. On the other hand, these humor samples to which were added 10% sodium citrate solution were subjected to the same treatments (KB-, PB-, PBK-, CKB- and NB-treatment) as mentioned in the author's previous report. In four horses (one case each of chronic, subacute and relapsed type and non EIA) siderocytes were recognized in the blood of jugular vein; bone marrow punctures were carried out at the tuber coxae, and 3 to 5 ml of the bone marrow punctate was investigated, anticoagulation and treatments as above described being employed. All experimental cases described above were dissected, and they were also submitted to histo-pathological examination. For the computation of the ratio of appearance of siderocytes and the decision of cytological varieties of siderocytes, the blood samples were treated by the methods (KB-, CKB-, PB- and NB-treatment) as described in the author's previous report; their hemograms were counted in the film of MAY-GIEMSA staining. The erythrocyte and leucocyte numbers were counted in the experimental cases which were observed for a long period, and leucocyte numbers only were examined in the blood of the splanchnic blood vessel. The histo-pathological examination was conducted by the methods described in the author's previous report.

RESULTS

(A) On the fluctuation of siderocytes in the blood of jugular vein

For the studies of ratio of appearance of siderocytes and proportion of the cytological varieties constructing siderocytes in the blood of jugular vein, three horses (cases 1, 2 and 3) which were affected artificially with EIA were under observation for periods of 433, 331 and 120 days respectively (each 91, 143 and 80 days after inoculation), and two naturally affected horses (cases 4 and 5) were observed for terms of 50 and 47 days.

Case 1 (Z 224, male, 2 years old)

Hematological and biopsical diagnoses were made by using spleen and liver punctures that were the non EIA. Siderocytes in the blood of jugular vein were recognized only rarely at the low ratio of appearance of about 1/50,000. Fever attack appeared from the 10th day after injecting intravenously 10 ml serum obtained from an affected horse. After that two typical fever paroxysms and a few irregular fever attacks occurred. Erythrocyte numbers had a tendency to diminish after inoculation. Leucocyte numbers exhibited no peculiar tendency, but they varied irregularly. The examination of hemogram showed remarkably that the proportion of monocytes increased gradually after inoculation, and a few reticulo-endothelial cells were recognized at rare intervals. A slight increase of lymphoid cells and small phagocytes was recognized temporarily in the initial stage of the infection. There were two weeks before the appearance of siderocytes began after the first fever attack. Following that siderocytes increased gradually in number, reaching a peak at the terminal stage of the third fever attack. After that, siderocytes decreased

gradually, then they increased again from the 65th day when the irregular fever attack began, exhibited the second peak at the stage of fever lysis and then they began gradual decrease. The proportions of cytological varieties constructing siderocytes showed predominant appearance of siderophagous neutrophilic leucocytes and siderophagous monocytes at the initial stage of the infection. Siderophagous neutrophilic leucocytes and siderophagous monocytes decreased gradually in accordance with development of the disease, whilst small siderophagous lymphoid cells and small siderophagocytes showed a tendency to increase contrary to the former blood siderocytes. This horse was slaughtered on the 91st day after inoculation and was diagnosed histo-pathologically as chronic type EIA. The splanchnic blood of this case was also examined (case 7).

Case 2 (Pr 3757, female, 2 years old)

This case was ascertained to be non EIA, clinically, hematologically and biopsically using spleen and liver punctions. Some irregular fever attacks were recognized; without exception the first typical fever paroxysm appeared on the 9th day after intravenous injection of 15 ml serum obtained from an affected horse. Erythrocyte numbers were recognized on a few occasions to be slightly decreased at the stages of fever attacks, but no remarkable changes were noted in over-all view of the course. The examination of hemogram showed that the proportion of monocytes increased while small lymphoid cells and small phagocytes increased slightly after inoculation. In other fractions, no remarkable changes were noted. Siderocytes were recognized usually at the ratio of about 1/100,000, but they increased gradually from the 4th day after inoculation. Particularly, siderocytes increased remarkably from the 20th day after inoculation, and they showed their peak of frequency at terminal stage of irregular fever attacks following the first fever attack. Thereafter siderocytes decreased gradually, but they showed temporarily a remarkable increase at 58th day, and they increased again remarkably from the 126th day after inoculation. The last two times of increase of siderocytes in ratio indicated no relation between the fever attack and erythrocyte numbers. By examination of the proportions of cytological varieties constructing siderocytes, it was recognized that siderophagous neutrophilic leucocytes and siderophagous monocytes occupy a great part at the stage showing the low ratio of appearance of siderocytes and that they decreased contrary to the increase of appearance of siderocytes. Accordingly, the proportions of siderophagous neutrophilic leucocytes and siderophagous monocytes relatively decreased gradually in accordance with development of the disease, while small siderophagous lymphoid cells and small siderophagocytes increased in parallel with the ratio of appearance of siderocytes. A few large siderophagocytes could be found only at the stage showing the high ratio of appearance of siderocytes. This horse was slaughtered on the 143rd day after inoculation and was diagnosed histo-pathologically as chronic type EIA.

Case 3 (Pr 3696, female, 18 years old)

This case was ascertained to be non EIA clinically, hematologically and biopsically. No siderocyte was recognized in the blood of jugular vein. Inoculations of tissue emulsion originating from mice to which the EIA virus passage was given were carried out three times at intervals of 19 and 17 days. Each irregular fever attack was recognized after some days from the first and second fever attacks, and some irregular fever attacks were

recognized after the third fever attack. It was ascertained to be EIA biopsically by the use of spleen and liver punctions on the 9th day after inoculation. Erythrocyte numbers decreased gradually from the 61st day after inoculation. No remarkable change was noted in the variations of leucocyte numbers. In hemograms, the proportions of monocytes, small lymphoid cells and small phagocytes were recognized to be slightly increased after inoculation. Siderocytes appeared from the 34th day after inoculation. The ratio of appearance of siderocytes varied slightly within a range of one and two per 10,000 leucocytes. In examination of the proportions of cytological varieties composing the siderocytes, siderophagous neutrophilic leucocytes occupied a great part in the initial stage of the infection; they decreased relatively in accordance with development of the disease. On the contrary, small siderophagous lymphoid cells increased in accordance with development of the disease. Siderophagous monocytes and small siderophagocytes, on the other hand, have no peculiar tendency through the whole course of the disease. This horse was slaughtered on the 80th day after the last inoculation; she showed signs histologically of chronic type EIA.

Case 4 (E 2103, female, 14 years old)

From the outset, clinical, hematological and biopsical examinations led to the suspicion that this horse was naturally affected with EIA, and also siderocytes were found in the blood of the jugular vein. The body temperature already, before inoculation, varied irregularly with some high fever attacks which seem to have been due to artificial factors, such as surgical operations, liver and spleen punctions and extirpation of submaxillary lymph nodes carried out on the previous day. The intravenous injection of 10 ml serum obtained from an affected horse on the 14th day after the beginning of the observation did not lead to remarkable variation of the body temperature. Erythrocyte and leucocyte numbers showed no peculiar over all tendency during the course of the disease. Erythrocytes were comparatively fewer with such a number as 4.86 to 5.46 millions. In the hemogram, the proportions of monocytes, small lymphoid cells and small phagocytes were recognized to be slightly increased after inoculation. The ratio of appearance of siderocytes was recognized to be remarkably increased each once before and after inoculation. In examination of the proportions of cytological varieties constructing siderocytes, siderophagous neutrophilic leucocytes and siderophagous monocytes were comparatively fewer, and they varied irregularly over the course. On the other hand, small siderophagous lymphoid cells and small siderophagocytes occupied the comparatively greater part, with the former decreasing in accordance with development of the disease, while small siderophagocytes increased to the contrary. The case was slaughtered on the 50th day after the beginning of the observation and was diagnosed histo-pathologically as relapsed type EIA.

Case 5 (E 2099, female, 15 years old)

This case was ascertained to be chronic type EIA by clinical, hematological and biopsical examinations. Siderocytes were recognized usually in the blood of jugular vein. The intravenous injection of 10 ml serum which was obtained from an affected horse was carried out on the 14th day after the beginning of the observation. Thenceforth, some irregular fever attacks were recognized. Erythrocyte numbers were recognized to be

remarkably decreased after inoculation, and leucocyte numbers seemed to be decreased in the same manner. Throughout the course, neutrophilic leucocytes occupied a small part. The proportions of monocytes, small lymphoid cells and small phagocytes in the hemogram were recognized to be slightly and gradually increased after inoculation. The ratio of appearance of siderocytes had a tendency to be gradually increased in accordance with development of the disease; that tendency was remarkable particularly after inoculation. In examination of the proportions of cytological varieties constructing siderocytes, siderophagous neutrophilic leucocytes and siderophagous monocytes were few before inoculation, while they increased after inoculation. On the other hand, small siderophagous lymphoid cells were recognized to be decreased after inoculation, whilst, to the contrary, small siderophagocytes and siderophagous monocytes have a tendency to be increased after inoculation. The case was slaughtered on the 47th day after the beginning of observation, and was diagnosed histo-pathologically as chronic type EIA.

(B) Systematic observation of siderocytes in the blood of the splanchnic blood vessels

Siderocytes in the circulating blood of the splenic vein, portal vein, hepatic vein, posterior vena cava, anterior vena cava, pulmonary artery, pulmonary vein, common carotid artery, jugular vein and anterior mesenteric vein were investigated on three cases of chronic type EIA and one case of relapsed type EIA; the mutual relations among the hemogram, histological findings and siderocytes were observed in the same manner. Those findings on the circulating blood should be described from the anterior mesenteric vein or splenic vein to the jugular vein in accordance with the direction of blood stream as above mentioned. Siderocytes in peritoneal fluid and lymph samples which were obtained respectively from the thoracic duct and abdominal cavity were examined, and the relation between these findings and the above one in the blood vessels was considered.

TABLE 1. *Siderocytes in Each Blood Vessel of Case 6*

SIDEROCYTE	BLOOD VESSELS				
	Splenic Vein	Portal Vein	Posterior Vena Cava	Pulmonary Vein	Jugular Vein
Siderocyte Numbers per 100,000 Leucocytes	133	100	50	10	7
	%	%	%	%	%
Large siderophagocyte	38.9	96.2	94.9	28.6	0
Small siderophagocyte	11.1	0	0	0	0
Small siderophagous lymphoid cell	13.9	0	0	0	0
Siderophagous neutrophilic leucocyte	11.1	0	0	14.3	66.6
Siderophagous monocyte	25.0	1.3	3.4	57.2	33.3

Case 6 (Pr 3715)

The case showed a low ratio of appearance of siderocytes (1/15,000). The ratio and cytological varieties of siderocytes which appeared in each blood vessel are indicated in table 1. The ratio of appearance of siderocytes was recognized to be slightly decreased as the sampling spots moved from the splenic vein to the portal vein, to be reduced by about one half in the posterior vena cava, and still more to be extremely decreased in the pulmonary vein.

In cytological examination of variations of siderocytes, large siderophagocytes showed high proportion in the splenic vein and those in the portal vein did still higher proportion irrespective of equivalent state in number. And they were recognized to be extremely decreased in the pulmonary vein, and to be completely lacking in the jugular vein. The proportions of small siderophagous lymphoid cells and small siderophagocytes were recognized to be gradually decreased in remove from the splenic vein to the posterior vena cava. The proportion of siderophagous monocytes was recognized to be comparatively high in the splenic vein, pulmonary vein and jugular vein. The proportion of siderophagous neutrophilic leucocytes was found to be comparatively higher in the jugular vein.

From the examination of hemograms, the lymphocytes, reticulo-endothelial cells and small lymphoid cells were found to occupy far greater proportion in the splenic vein than in any other of the blood vessel, and they had a similar tendency in the portal vein. Large reticulo-endothelial cells were recognized to be decreased in the posterior vena cava following the portal vein through the liver. The proportions of lymphocytes, small lymphoid cells and reticulo-endothelial cells were recognized to be increased again in the pulmonary vein following the pulmonary artery which unites the anterior vena cava into which the lymph flows through the thoracic duct.

As signs of chronic type EIA, remarkable proliferation of small lymphoid cells and slight proliferation of reticular cells were observed in the spleen and lymph nodes; swelling and proliferation of reticulo-endothelial cells were observed in the liver. Findings of hemosiderosis showed the existence of a large number of granular hemosiderins in the above proliferous reticulo-endothelial cells of the liver and spleen, and mild hemosiderosis was recognized in the lung.

Case 7 (Z 224)

The case showed a high ratio of appearance of siderocytes (1/1,000). The ratio and cytological variation in siderocytes which appeared in each blood vessel are indicated in table 2.

The ratio of appearance of siderocytes was recognized to be slightly decreased in remove from the splenic vein to the portal vein, to be still more decreased towards the hepatic vein, and posterior vena cava; on the other hand the ratio was again increased in the anterior vena cava and pulmonary artery. However, the ratio of appearance of siderocytes in the blood of jugular vein showed an extremely low value, and the ratio in the anterior mesenteric vein was more decreased.

In examination of the proportions of cytological varieties constructing siderocytes, large siderophagocytes occupied about one-half of siderocytes in the splenic vein, increase in the proportion was recognized in the portal vein and hepatic vein, but no change in

TABLE 2. Siderocytes in Each Blood Vessel of Case 7

SIDEROCYTE	BLOOD VESSELS								
	Splenic Vein	Portal Vein	Hepatic Vein	Posterior Vena Cava	Anterior Vena Cava	Pulmonary Artery	Jugular Vein	Anterior Mesenteric Vein	
Siderocyte Numbers per 100,000 Leucocytes	133	200	175	125	200	175	100	50	
	%	%	%	%	%	%	%	%	%
Large siderophagocyte	60.0	72.0	74.3	46.3	45.6	34.6	0	0	
Small siderophagocyte	10.0	10.9	6.4	14.7	7.8	17.2	18.8	35.0	
Small siderophagous lymphoid cell	16.7	7.9	6.4	14.7	25.6	20.6	43.6	40.0	
Siderophagous neutrophilic leucocyte	10.0	6.2	8.3	7.3	6.6	7.0	18.8	5.0	
Siderophagous monocyte	3.3	3.0	4.6	17.0	14.4	20.6	18.8	20.0	

number of cells was recognized. The proportion of the cells gradually decreased according distance increased from the hepatic vein; no large siderophagocytes were recognized in the jugular vein. The proportion of small siderophagocytes was recognized to be gradually increased from the splenic vein to the jugular vein relatively, but no change in number of cells was recognized. The proportion of small siderophagous lymphoid cells occupied a quarter of siderocytes in the splenic vein was studied; it was recognized to be extremely decreased in order of the portal vein, hepatic vein and posterior vena cava, while it was remarkably increased in the anterior vena cava into which the lymph flows through the thoracic duct. The cells occupied a half of siderocytes in the blood of jugular vein and anterior mesenteric vein. No remarkable change of proportion of siderophagous neutrophilic leucocytes was recognized in remove from the hepatic vein to the pulmonary vein, but the proportion of the cells was recognized to be extremely increased in the jugular vein. Siderophagous neutrophilic leucocytes, on the other hand, were recognized to be very few in the anterior mesenteric vein. The proportion of siderophagous monocytes did not show remarkable change in remove from the splenic vein to the hepatic vein; that proportion showed gradual increase with increase in distance from the posterior vena cava.

By examination of the hemogram, a large number of reticulo-endothelial cells, small phagocytes and small lymphoid cells were recognized in the splenic vein. But reticulo-endothelial cells were recognized to be reduced by one-half in the posterior vena cava, and also small siderophagocytes and small lymphoid cells have a tendency to be gradually decreased similarly. However reticulo-endothelial cells, small phagocytes and small lymphoid cells were recognized to be extremely increased in the anterior vena cava, and

to be decreased in the pulmonary vein. In the jugular vein, no reticulo-endothelial cells were recognized, whilst small lymphoid cells were recognized to be remarkably decreased.

This case was diagnosed as chronic type EIA histo-pathologically. Remarkable proliferation of small lymphoid cells and proliferation of reticulo-endothelial cells were recognized in the spleen and lymph nodes; proliferation of aggregate nodules of small lymphoid cells and swelling and proliferating endothelial cells were recognized in the liver.

Investigation of hemosiderosis revealed the appearance of a few hemosiderin granules in the above proliferating reticulo-endothelial cells of the liver, spleen and lymph nodes.

TABLE 3. *Siderocytes in Each Blood Vessel of Case 8*

SIDEROCYTE	BLOOD VESSELS									
	Splenic Vein	Portal Vein	Hepatic Vein	Posterior Vena Cava	Anterior Vena Cava	Pulmonary Artery	Pulmonary Vein	Common Carotid Artery	Jugular Vein	
Siderocyte Numbers per 100,000 Leucocytes	2,940	2,600	1,530	1,360	1,590	1,280	870	1,010	600	
	%	%	%	%	%	%	%	%	%	%
Large siderophagocyte	27.2	27.7	28.9	14.9	5.3	11.2	3.5	2.0	0	
Small siderophagocyte	35.4	40.0	43.3	43.9	29.8	24.3	28.7	28.7	24.1	
Small siderophagous lymphoid cell	27.2	22.3	23.3	14.0	53.2	48.6	48.3	42.6	35.8	
Siderophagous neutrophilic leucocyte	4.8	7.7	3.3	21.9	8.5	11.2	18.3	21.8	35.0	
Siderophagous monocyte	5.5	2.3	1.1	5.3	3.2	4.7	1.1	4.9	5.0	

Case 8 (Z 232)

This case showed a comparatively high ratio of appearance of siderocytes (1/500). The ratio and cytological varieties of siderocytes which appeared in each blood vessel are indicated respectively in table 3. The ratio of appearance of siderocytes was not recognized to be changed in remove from the splenic vein to the portal vein, the appearance of siderocytes was recognized to be reduced in the hepatic vein, and thence no remarkable change of the ratio was recognized in remove towards the pulmonary vein. But extreme reductions in the ratio were recognized in each remove from the pulmonary artery and common carotid artery to the pulmonary vein and jugular vein.

In examination of the proportions of variations in cytological nature of siderocytes, large siderophagocytes occupied about one-third of the siderocytes in the splenic vein, thence were recognized gradual decrease according to increase in the distance; particularly there were recognized extreme decrease in remove from the pulmonary artery to the pulmonary vein; siderocytes were unable to be found in the jugular vein. The proportion of small siderophagocytes was two-fifths of the total siderocytes in the splenic vein; the

proportion showed a tendency to be gradually decreased in remove from the splenic vein, whilst it had a tendency to be gradually decreased in remove from the splenic vein to the jugular vein. The proportion of small siderophagous lymphoid cells tended to be gradually decreased in remove from the splenic vein to the posterior vena cava, while it occupied about one-half of siderocytes in the anterior vena cava. Thence it again had a tendency to be gradually decreased in remove towards the jugular vein. The proportion of siderophagous neutrophilic leucocytes was generally recognized to be small, but it had a tendency to be generally increased in remove from the splenic vein to the posterior vena cava; on the other hand it was recognized to be small in the anterior vena cava, and thence remarkable increase was recognized in remove towards the jugular vein. The proportion of siderophagous monocytes was recognized to be small on the whole, and its fluctuation did not show a particular tendency by blood vessel.

On the other hand, a large number of small siderophagous lymphoid cells and small siderophagocytes were recognized in the lymph of the thoracic duct. A large number of small siderophagocytes and large siderophagocytes were found in the peritoneal fluid.

In the examination of hemograms, a large number of reticulo-endothelial cells, small phagocytes and small lymphoid cells were recognized in the splenic vein, and thence showed gradual decrease in remove towards the posterior vena cava. A number of small lymphoid cells and small phagocytes were recognized in the anterior vena cava, and thence showed remarkable decrease in remove towards the jugular vein. No reticulo-endothelial cells were found in the jugular vein.

The diagnosis was chronic type EIA on the basis of histological findings as follows: remarkable proliferation of small lymphoid cells and significant proliferation of reticulo-endothelial cells were recognized in the spleen and lymph nodes, and a number of aggregate nodules of small lymphoid cells and swelling and proliferating endothelial cells were recognized in the liver.

In the examination of hemosiderosis, a few hemosiderin granules were recognized in the above proliferating reticulo-endothelial cells in the liver, spleen and lymph nodes. Remarkable hemosiderosis was recognized in the proliferating large phagocytes and small phagocytes of the milky spots of the greater omentum. Hemosiderosis could hardly be recognized in the bone marrow.

Case 9 (Z 231)

The case showed a low ratio of appearance of siderocytes (16/100,000). The ratio and cytological varieties of siderocytes as they appeared in each blood vessel are indicated in table 4. In the ratio of appearance of siderocytes there was recognized gradual decrease in remove from the splenic vein to the jugular vein.

Examination of the proportions of cytological varieties constructing siderocytes showed that large siderophagocytes provided about one-half of the siderocytes in the splenic vein and portal vein; they showed extreme decrease in remove to the hepatic vein, thence they tended to be gradually decreased, and they quite disappeared in the jugular vein. On the other hand, small siderophagocytes in remove from the splenic vein to the jugular vein showed mild decline in number as compared with the decrease of large siderophagocytes, but their proportion seemed to be increased in remove towards the jugular vein. Not

TABLE 4. *Siderocytes in Each Blood Vessel of Case 9*

SIDEROCYTE	BLOOD VESSELS									
	Splenic Vein	Portal Vein	Hepatic Vein	Posterior Vena Cava	Anterior Vena Cava	Pulmonary Artery	Pulmonary Vein	Common Carotid Artery	Jugular Vein	
Siderocyte Numbers per 100,000 Leucocytes	330	270	96	88	32	30	37	37	16	
	%	%	%	%	%	%	%	%	%	%
Large siderophagocyte	45.6	48.1	29.1	9.0	18.8	3.3	2.7	0	0	
Small siderophagocyte	42.4	40.7	54.1	68.2	56.3	70.0	59.5	60.1	66.7	
Small siderophagous lymphoid cell	0	0	0	0	0	0	0	0	0	
Siderophagous neutrophilic leucocyte	6.0	7.4	8.4	9.1	6.2	6.7	13.5	30.0	25.0	
Siderophagous monocyte	6.0	3.7	8.4	13.6	18.7	20.0	24.3	10.0	8.4	

even one small siderophagous lymphoid cell was found. In the proportions of siderophagous neutrophilic leucocytes and siderophagous monocytes, remarkable difference could not be recognized blood vessel by blood vessel. A few siderocytes constructed of only large siderophagocytes were recognized in the lymph of thoracic duct, and no siderophagocyte was found in the peritoneal fluid.

In the examination of the hemograms, a number of siderophagous neutrophilic leucocytes were recognized as compared with lymphocytes in all the blood vessels, and also a few large lymphoid cells were found. A large number of reticulo-endothelial cells and small phagocytes were recognized in the splenic vein, and thence they had a tendency to be gradually decreased in remove toward the jugular vein. But a number of small phagocytes were recognized in the anterior vena cava.

The diagnosis was subacute type EIA from the histological findings as follows: remarkable proliferation of reticulo-endothelial cells and large lymphoid cells and slight proliferation of small lymphoid cells were recognized in the spleen, liver and lymph nodes; swelling and proliferating endothelial cells and a number of aggregate nodules consisting of large lymphoid cells and reticulo-endothelial cells were recognized in the liver.

Considerable hemosiderosis was recognized in the spleen, liver and lymph nodes. A few hemosiderin granules were found rarely in reticular cells of the milky spots of the greater omentum. Remarkable hemosiderosis was recognized generally in the bone marrow. The bone marrow punctate of this case was also provided for examination (case 11).

The above data which could be expressed by numbers such as the ratio of appearance of siderocytes and the proportions of each cytological variety of siderocytes were analyzed

statistically on the basis of siderocytes numbers in 1 ml of blood, the findings as mentioned above were confirmed to be significant.

Siderocytes in the bone marrow

In order to know how the bone marrow related to the appearance of siderocytes, cytological and histological examinations were carried out as follows. For four cases in which the appearance of siderocytes was recognized in the blood of jugular vein (each one case chronic, subacute and relapsed type EIA and non EIA), cytological examination of siderocytes in the bone marrow was carried out, and the relationship among siderocytes in the blood of jugular vein and siderocytes and hemosiderosis in the bone marrow was investigated.

Case 10 (Z 230)

The case showed a low ratio of appearance of siderocytes in the blood of jugular vein (1/10,000); siderophagous monocytes and siderophagous neutrophilic leucocytes were recognized in the examination of the proportions of cytological varieties constructing siderocytes. Histological changes proved the chronic type EIA.

Case 11 (Z 231)

A low ratio appearance of siderocytes (12/100,000) was recognized in the blood of jugular vein. The cytological varieties constructing siderocytes consisted of all cytological varieties except small siderophagous lymphoid cells. Histo-pathological changes in the animal proved the subacute type EIA.

Case 12 (Z 225)

This case showed high ratio of appearance of siderocytes in the blood of jugular vein (1/750). The cytological varieties constructing siderocytes included various varieties except small siderophagocytes. Histo-pathological changes proved the relapsed type EIA.

Case 13 (Z 226)

Low ratio of appearance of siderocytes (1/15,000) was found in the blood of jugular vein. The cytological variations in constituent siderocytes were only small siderophagous lymphoid cells. In histological examination, the diagnosis was reached as non EIA.

The cytological varieties constructing siderocytes in the bone marrow punctate were large siderophagocytes and small siderophagocytes.

By histological examination of the bone marrow, hemosiderin of the granular and diffuse types was recognized generally in reticulo-endothelial cells and small phagocytes.

DISCUSSION

In the study of the fluctuation of siderocyte numbers in the blood of jugular vein of many artificially affected horses, ISHII et al. (1940) reported as follows: the appearance of siderocytes happens at the same time as the febrile attack or after a few days from the beginning of febrile attack in general, while in a few cases it begins within the latent period. MORITA and ICHIOKA (1957) reported that the first appearance of siderocytes happens with the first febrile attack in

the inoculation of abundant EIA virus, but it is recognized as the first symptom of the injection in the inoculation of a small amount of EIA virus because no remarkable febrile attack is recognized.

In the present study, the first appearance of siderocytes in the blood of jugular vein was found in the latent period of case 2 (Pr 3757), after the crisis of the first fever attack in case 1 (Z 224) and on the 34th day after the inoculation in case 3 (Pr 3696). No clear reason for these phenomena seems to be considerable excepting the virulence and quantity of the virus and the individual differences of the horse body. The fact that the highest ratio of appearance of siderocytes in the blood of jugular vein of cases 1 and 2 happened after the crisis of fever, coincides with the finding of ISHII and his coworkers' report (1940). In cases 4 and 5 (both natural) and case 3 (artificial inoculation with the tissue emulsion originated from mice to which the EIA virus passage were given), on relationship between the ratio of appearance of siderocytes and the febrile attack was recognized. It is clear that these findings were due to the particular reasons that cases 4 and 5 had affected naturally before the inoculation and that case 3 was affected with tissue emulsion originated from mice to which the EIA virus passage was given. In the finding of artificially affected cases, the proportions of cytological varieties constructing siderocytes showed predominant appearance of siderophagous neutrophilic leucocytes and siderophagous monocytes at the first stage with low ratio of appearance of siderocytes; the proportions of small siderophagous lymphoid cells and small siderophagocytes increased together with the increase of the ratio of appearance of siderocytes in accord with the development of the disease. In the natural cases, the small siderophagous lymphoid cells provided a great proportion of the siderocytes from the outset; the finding coincides with the finding of the prolonged course in the artificially affected cases. Those findings seem to indorse YAMAGIWA's reports^{36,39)} that the chronic type EIA takes a chronic course from the initial stage showing chronic type lesions mainly such as small lymphoid cellular reaction in the lympho-reticular tissue. Therefore, the author is unable to support ISHII's opinion⁷⁾ that the demonstration of siderocytes is difficult or impossible at the stages of lymphoid cellular and fibrillar reaction. In case 4 which was again inoculated artificially with EIA virus after the natural infection, the ratio of appearance of siderocytes showed sudden increase some days before the autopsy; the construction of cytological varieties of siderocytes changed gradually to that of relapsed type EIA in accordance with development of the disease, and the diagnosis was made as relapsed type EIA on basis of the histo-pathological examination. Those facts are very interesting as manifestations that the lesions had changed from chronic type to relapsed. Since the ratio of appearance of siderocytes in case 5 increased

slightly after inoculation and the change of cytological construction of siderocytes was similar to it of case 4, case 5 which was accorded the same treatment seems to have shown the vital reaction similar to that of the relapsed type EIA after the inoculation.

By the observation of the ratio of appearance of siderocytes in the blood of the splanchnic blood vessels, the extreme decrease of their ratio was recognized to remove from the pulmonary artery to the pulmonary vein and from the common carotid artery to the jugular vein. That fact supported ISHII's opinion¹³⁾ which the siderocytes would be stuffed physically in the pulmonary capillaries. According to observation of the cytological varieties of siderocytes in the blood of the splanchnic blood vessels of case 6 (Pr 3715) which showed low ratio of appearance of siderocytes in the jugular vein and the chronic type EIA lesion, large siderophagocytes which occupied a large part in the splenic vein, portal vein and posterior vena cava, exhibited remarkable decrease in the pulmonary vein, and they disappeared in the jugular vein. The proportions of small siderophagocytes and small siderophagous lymphoid cells showed extreme decrease according to increase in the distance from the splenic vein. The proportion of siderophagous monocytes was low in general; they were various and disagreeing in each blood vessel. A number of siderophagous neutrophilic leucocytes were recognized in the jugular vein. By the same observation of case 7 (Z 224) and case 8 (Z 232) which showed comparatively higher ratio of appearance of siderocytes in the blood of jugular vein and the chronic type EIA lesion, large siderophagocytes provided a large part of the siderocytes in the same manner as the previous case (Pr 3715) in a zone from the splenic vein to the hepatic vein, thence they decreased gradually according to increase in the distance from those blood vessels, and they were not recognized at all in the jugular vein. Siderophagous neutrophilic leucocytes displayed gradual increase with remove towards the jugular vein, and siderophagous monocytes showed increase in the posterior vena cava and pulmonary artery. According to the findings as above mentioned, it is supposed that nearly large siderophagocytes would be stuffed in the capillaries and a few siderophagous neutrophilic leucocytes and siderophagous monocytes of the native blood cells would assume the shape of siderocytes in the blood stream. In the most of large siderophagocytes, their cytoplasm was swollen with vacuoles and hemosiderin granules, and their nuclei showed metamorphosis and the reduction of affinity to alum-carmin dye. The above findings show that those cells stuff and collapse (phagolysis) before long. In the present finding, hemosiderin granules which were discharged from the collapsed large siderophagocytes are recognized. For that reason, it is supposed that those hemosiderin granules would be captured by the cells with phagocytosis such as siderophagous neutrophilic leucocytes,

siderophagous monocytes, small lymphoid cells and small phagocytes.

In the initial stage of the infection of EIA, siderophagous neutrophilic leucocytes and siderophagous monocytes of native siderophagous blood cells were recognized to appear in the blood stream before the appearance of histogenic siderocytes in the blood of jugular vein. The above fact seems understandable by reason that the blood cells with phagocytosis turn into siderocytes, because the change of iron metabolism in the blood and tissue (as the increase of ferritin in the blood stream and tissue) happens before small histogenic siderocytes are discharged in the blood; however, those cells proliferate in the tissue. Therefore, siderophagous blood cells and histogenic siderocytes originally differ entirely in the cytogenesis. Since small siderophagocytes were recognized to be comparatively more in number in the splenic vein and anterior vena cava, the spleen and thoracic duct where the lymph flows together from the lymph nodes of the whole body are considered to be the main providers of such cells. However, small siderophagocytes were recognized not so much by the difference of proportion of siderocytes in each blood vessel. They were recognized in the peritoneal fluid—the milky spots of the greater omentum—and the bone marrow etc. That above fact is interesting as a finding to indorse the author's opinion expressed in the previous report that small siderophagocytes originate from the reticular tissue in a wide sense. As still more interesting findings in cases 7 and 8, small siderophagous lymphoid cells exhibited gradual decrease in remove from the splenic vein to the posterior vena cava; they increased remarkably in the anterior vena cava, and they occupied a comparatively greater part of the siderocytes in the jugular vein. On the other hand, a large number of small siderophagous lymphoid cells and a considerable number of small siderophagocytes were recognized in the lymph of the thoracic duct of case 8. That finding has an important significance to prove the finding that small siderophagous lymphoid cells which originated from the lymph nodes of the whole body flowed into the anterior vena cava by way of the thoracic duct. That is to say, it is a fortunate result beyond the author's expectation that the above findings were obtained in the present study. The findings are considered to throw light again upon the matrix of small siderophagous lymphoid cells which were classified by the author in the previous report. That fact is indorsed by the YAMAGIWA's and his coworkers' opinion^{36,39)} that the chronic type EIA takes a chronic course showing chronic type lesions mainly such as small lymphoid cellular reaction in the lymph-reticular tissue from the initial stage. Therefore, by their opinion and the author's new findings, it was confirmed as an established fact that small lymphoid cells occupied the main part of cytological varieties constructing siderocytes in the blood of jugular vein of chronic type EIA cases. In case 9 (Z 231) which showed low ratio

of appearance of siderocytes in the blood of jugular vein and the subacute type EIA lesion, large siderophagocytes, siderophagous neutrophilic leucocytes and siderophagous monocytes in the blood of splanchnic blood vessels have a tendency similar to the finding in case 6, but it differs entirely from the finding of above three cases with the chronic type EIA lesions that no small siderophagous lymphoid cells were recognized in the blood of whole circulatory system of case 1. On the contrary, small siderophagocytes showed a tendency similar to the finding of cases 7 and 8 with the high ratio of appearance of siderocytes in the blood of jugular vein and the chronic type EIA lesion. The finding is able to be explained easily because the reticulo-endothelial cells, small phagocytes and large lymphoid cells are mainly proliferated cells in the subacute type EIA lesion.

These findings lead the present author to the conclusion that the cytological varieties of siderocytes in the blood of jugular vein are the expressions of the lesion in the tissue with close relation to the histological lesion.

SUMMARY AND CONCLUSIONS

Siderocytes in the blood of jugular vein were investigated in five cases for a long period, and the splanchnic blood vessels were observed systematically in four cases. The findings may be summarized as follows:

1) In artificially affected horses, a number of siderocytes in the blood of the jugular vein were demonstrated just at or after the crisis of fever. According to the observation of the cytological varieties of the appeared siderocytes, the proportions of siderophagous neutrophilic leucocytes and siderophagous monocytes occupied a large part in the first stage when the ratio of appearance of siderocytes was comparatively lower; both their proportions declined gradually with the development of the disease, while the proportions of small siderophagous lymphoid cells and small siderophagocytes showed gradual increase.

In the cases where the naturally affected horses were reinoculated, the ratio of appearance of siderocytes showed no relation to the fever. According to the observation of the cytological varieties of appearance of siderocytes, the proportion of small siderophagous lymphoid cells occupied a large part before the reinoculation, they decreased gradually in the course of time after the reinoculation, and the proportion of small siderophagocyte showed relative increase.

2) In the splanchnic blood vessels, the ratio of appearance of siderocytes demonstrated extreme decrease following the passage of the capillaries in remove from the pulmonary artery and common carotid artery to the pulmonary vein and jugular vein; in the cases with high ratio of appearance of siderocytes in the blood of jugular vein and the chronic type EIA lesion, the proportion of large siderophagocyte occupied a large part in the splenic vein and hepatic vein.

However, from the most probable fact that siderocytes were stuffed in the capillaries, they had decreased or disappeared in the pulmonary vein and jugular vein. Small siderophagocytes in each blood vessel show a tendency similar to the above cells, but no remarkable difference in proportion in each blood vessel was recognized. The proportion of small siderophagous lymphoid cells tends gradually to decrease in remove from the splenic vein to the posterior vena cava, while remarkable increase was demonstrated in the anterior vena cava joining the thoracic duct with a large number of small siderophagous lymphoid cells and small siderophagocytes constructing the main part of siderocytes. The proportions of siderophagous neutrophilic leucocytes and siderophagous monocytes failed to show remarkable difference in remove from the splenic vein to the posterior vena cava, while relative increase was demonstrated in remove from the pulmonary artery to the jugular vein. In the cases with the low ratio of appearance of siderocytes in the jugular vein, the proportions of large siderophagocytes, small siderophagocytes and small siderophagous lymphoid cells remarkably decreased or disappeared with increasing distance from the splenic vein. In the case with subacute type EIA lesion, it is different from the finding of the cases with the chronic type EIA lesion that small siderophagous lymphoid cells were not found in the blood. The kinds of siderocytes in the peritoneal fluid and in the bone marrow punctuate were only large and small siderophagocytes.

The proportions of each cytological variety of the appeared siderocytes in each blood vessel have relation to the hemogram of each blood vessel.

In accordance with the findings outlined above, small phagocytes and small lymphoid cells which originated from the reticular tissue and lympho-reticular tissue respectively are considered to turn into small siderophagocytes and small siderophagous lymphoid cells respectively. Also histological findings which indorse the above facts were recognized.

3) The author is led to the conclusion that the siderocytes appearing in the blood of the jugular vein are an expression of the EIA lesion. That is to say, siderophagous neutrophilic leucocytes and siderophagous monocytes originated from the native blood cells, and they appear from the initial stage to the terminal stage in response to the changes of the vital functions. Large siderophagocytes, small siderophagocytes and small siderophagous lymphoid cells, on the other hand, originated from the histogenic cells, and they were proved to appear as an expression of the histological lesion having close relation to the histological reaction.

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