CYTOCHEMICAL STUDIES ON THE LESION OF EQUINE INFECTIOUS ANEMIA VII. ESPECIALLY ON THE ENZYME HISTOCHEMISTRY OF RES IN THE LIVER AND SPLEEN

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VII. ESPECIALLY ON THE ENZYME HISTOCHEMISTRY OF RES IN THE LIVER AND SPLEEN

By

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Introduction

The pathology of equine infectious anemia (EIA) has been the subject of extentive investigation for sixty years. Itikawa (1–6), one of the present authors, has six papers in cytochemical studies on the lesion of EIA. His first research was on the activation of reticulo-endothelial system (1), followed by (2) the nature of so-called lymphoid cells, (3) the degeneration of glycogen in hepatic cells, (4) the extracellular hemosiderosis of serous fluid in the tissue, (5) the formation of crystal consisting of polysaccharide-iron-calcium complex in the trabecular artery (6) the relationship between microincineration and emission spectrograph in hepatic and splenic hemosiderosis in the Bull. N.I.A.H. on 1958–1960. In addition Itikawa reported on such related subjects, as the interrelationship between hepatic and splenic hemosiderosis in EIA (7), on the cytochemical observation of EIA with liver-needle biopsey materials (8, 9), on the cytochemistry of Altara-Serra-Guarini's antigen for EIA-complement fixation test (10), on dehydrogenase in the liver and spleen of EIA (11–12), and on dehydrogenase of the white blood corpuscles in the jugular vein and hepatic artery (2, 13).

The pathogenesis of EIA depends, accordingly to Dobberstein (14), upon three reactions of the wall of blood vessels caused by the stimulation of EIA virus,

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namely, histiocytic (phagocytosis), lymphoidcytic (non-phagocytosis) and fibrinous (precollagenous fiber formation). Histiocytes and lymphoid cells originate from the cells of the capillary walls and bulid up the reticulo-endothelial system (RES) with the endothelium of hepatic sinuses, pulp cells and reticular cells of the spleen and lymph nodes, all of which are considered to be derived from the undifferentiated cells on the capillary wall. The virus of EIA stimulates chiefly RES and mesenchym of the undifferentiated capillary wall in the resting state and causes proliferation of cells on the capillary wall in each organ.

According to Itikawa (1), cells found in the activated endothelium of the capillary, as well as reticular cells, are pyroninophilic and digested by ribonuclease, and the cells observed in phagocytosis and in the phagocytosing stage are not pyroninophilic. Ribose nucleic acid (RNA) was found in the cytoplasm of the activated cells, as was reported by Szanto and Popper (15) and Itikawa (1), by means of the ultraviolet microscope at the wave length of 2650Å. Mitotic division in the intrasinusoidal proliferation shown by Feulgen's DNA reaction was not only thrombosis caused by immigrating cells but proliferation in that field of activation (1). The cellular groups containing positive granules showed the presence of dehydrogenase, which was found in the same places as lymphoid cell infiltration (1). Although the activation of mesenchymal cells according to Dobberstein (14) accepted no detailed description nor conclusive evidence is available for it. Itikawa (1) described histo- and cytochemically, the nature of lymphoid cells, and activated RES with RNA containing much succinic dehydrogenase.

A method of microscopic detection of succinic dehydrogenase in the tissue of ditetrazolium salts was established by Seligman (16), and thereafter Itikawa et al. published many papers on the application and modification of his method (2, 11, 12, 13, 17, 18, 19, 20). Itikawa (13) established a new method using free cells in the peripheral and visceral blood for qualitative and quantitative analysis. The author (13) recognized morphologically and quantitatively the increase in the number of large mononuclear leucocytes containing much succinic dehydrogenase among white blood corpuscles derived from the jugular vein and the hepatic artery of horses affected with EIA, and also that the origin of lymphoid cells observed in EIA (2) consists in RES, and is worthwhile in distinguishing the activation of the cells with RNA and dehydrogenase from that of the cells with little RNA and dehydrogenase.

According to the degree of hemosiderin deposition in the liver and spleen, the author (7) distinguished hypersiderosis (H), siderosis, hyposiderosis (h) and asiderosis, and consolidated siderosis to hypersiderosis and asiderosis to hyposiderosis. Accordingly, the author has classified the following four types in the interrelationship between hepatic and splenic hemosiderosis: Type I (HH), hepatic hypersiderosis and splenic hypersiderosis, type II (Hh), hepatic hyposiderosis and splenic hyposiderosis, type III (hH), heaptic hyposiderosis and splenic hypersiderosis, and type IV (hh), hepatic hyposiderosis and splenic hyposiderosis. The author (7)

studied the livers and spleens of 508 cases consisting of 345 cases of EIA and 163 cases of non-EIA. In the 345 cases of EIA, type I (HH) was found in 51%, type II (Hh) in 26%, type III (hH) in 6% and type IV (hh) in 17%. On the other hand in 163 cases of non-EIA, type I (HH) was found in 1%, type II (Hh) in 12%, type III (hH) in 18% and type IV (hh) in 69%.

The present study deals with the occurrence of enzymatic activities in RES of the liver and spleen of EIA contained in type I (HH), type II (Hh), type III (hH) and type IV (hh) by the classification of the interrelationship between heaptic and splenic hemosiderosis. Recent biochemical studies of the metabolism of the red blood corpuscles, according to Nakao (21), depends upon the following points, 1) the dependence upon glucose in the energy of the erythrocytes, 2) the production of adenosin-3-phosphate (ATP) by glycogenolysis, 3) maintenance of erythrocyte form by the energy of ATP, 4) oxidation and reduction of nicotinamide-adenine dinucleotide (NAD) in the Embden-Meyerhof's cycle, 5) production of nicotinamide-adenine dinucleotide phosphate (NADPH) by the reduction NADP in the pentose-phosphate pathway, 6) importance of NADPH as coenzyme for the formation of reduced glutathione protecting SH base of essential protein in the erythrocytes, 7) ATP as an energy for the active transport of cation into the erythrocytes, 8) principal role of ATP-ase in the erythrocytic membrane, 9) abscence of glycogen-synthetized system such as glycogen in the mature erythrocytes, and 10) depression of glucose-6-phosphate (G-6-P) dehydrogenase, decrease of production of NADPH, reduced gluthatione and glyceryl aldehyde 3 phosphate-dehydrogenase.

Furthermore, in studying the enzymatic metabolism in the cells, it might be important to investigate the following enzymes: 1) acid phosphatase for nucleic acid metabolism and phagocytosis and pinocytosis by the lysosomal particles in the activation of the cell membrane, 2) alkaline phosphatase, α-glycerophosphate dehydrogenase, G-6-P dehydrogenase, NAD-diaphorase, NADP-diaphorase for glycogenolysis, 3) lactate dehydrogenase related to amino acid metabolism such as tryptophane or phenylalanin, 4) testosteron-dehydrogenase and estrogen-dehydrogenase related to steroid metabolism, 5) monoamine oxidase (MAO) hydrolysed amine to aldehyde and ammonia in the protein decomposition and 6) succinate dehydrogenase, NAD- and NADP-diaphorase (as an electron-transportation system) for the cellular respiration.

Materials and Methods

The materials studied were 22 livers and spleens which had been taken from 7 EIA and 10 non-EIA horses killed at the Shibaura Slaughterhouse in Tokyo, 3 EIA horses at Yamagata Prefecture, and 2 EIA horses at Miyagi Prefecture. The total fresh livers and spleens from these EIA or non-EIA horses were collected just after slaughtering, and cut with the cryostat microtome at 25°C below zero, and stained with the various enzyme-histochemical procedures. Histochemical

procedures used were Gomori's alkaline phosphatase (22), Rutenberg's acid phosphatase (23), Padykula's adenosintriphosphatase (24), Nachlas's succinic dehydrogenase (25), lactic dehydrogenase (26), Cohen's TPNH-diaphorase (27), Cohen's DPNH-diaphorase (27), Burston's glucose-6-phosphate dehydrogenase (28), Glenner's MAO (29), and Wattenburg's steroid dehydrogenase (30).

Results

(1) *Enzyme-histochemical changes in the hepatic cells of EIA- and non EIA-horses*

The interrelationship between the intensity of hepatic lesion and the distribution of various enzymes in the hepatic cells is indicated in Table 1. According to Itikawa's classification (7) of the interrelationship between hepatic and splenic hemosiderosis, there are 4 types: Type I (HH), type II (Hh), type III (hH) and type IV (hh). Observing the intensity of hemosiderosis in the liver (hepatic cells and hepatic RES) and spleen (trabecule, follicle, and sinuses), in the 12 cases of EIA,

Table 1. Distribution of various enzymes in the

EIA or non-EIA	Type	Name of horses	Type of hepatic and splenic hemosiderosis	Hemosiderosis					Type of hepatic siderosis
				Liver		Spleen			
				Hepatic cells	Hepatic RES	Trabecule	Splenic pulp	Sinuses	
EIA	I	Y 1	HH	⫲	⫲	−	+	⫲	D
		S 1	HH	⫲	⫩	+	⫩	⫩	N
		S 2	HH	⫲	⫲	−	⫲	⫩	N
		S 3	HH	⫲	⫲	+	+	⫩	N
		S 4	HH	⫲	⫲	⫩	⫲	⫩	N
		S 5	HH	⫲	⫲	⫲	⫩	⫩	N
		S 6	HH	⫲	⫲	+	⫩	⫲	N
		S 7	HH	⫩	⫩	−	−	⫩	D
	II	M 1	Hh	⫩	⫩	⫩	−	−	D
		Y 2	Hh	⫩	⫩	⫩	−	−	D
	III	Y 3	hH	+	⫩	−	−	⫩	D
	IV	M 2	hh	+	+	−	−	−	D
non-EIA	III	S 87	hH	−	−	⫩	−	⫩	−
		S 89	hH	−	−	+	−	⫩	−
		S 91	hH	−	−	−	−	⫲	−
		S 107	hH	−	−	−	+	⫲	−
		S 168	hH	−	−	⫩	−	⫲	−
		S 190	hH	−	−	−	−	⫲	−
	IV	S 143	hh	−	−	−	−	+	−
		S 189	hh	−	−	−	−	+	−
		S 191	hh	−	−	−	−	+	−
		S 207	hh	−	−	−	−	+	−

type I was found in 67%, type II in 17%, type III in 8% and type IV in 8%; and on the other hand in 10 cases of non-EIA, type I was found in 0%, type II in 0%, type III in 60% and type IV in 40%.

a) The activities of alkaline phosphatase, acid phosphatase, ATP-ase, succinic DHG, lactic DHG, G-6-P DHG, NADH-diaphorase, NADPH-diaphorase, testosteron-DHG, and estradiole-DHG in the hepatic cells of type I (HH) of EIA were stronger than those of α-glycerophosphate DHG and MAO. In comparison with type III (hH) and IV (hh) of non-EIA there were great differences between the decrease of acid P-ase in the nodular hemosiderosis of type I (HH) of EIA.

b) The activities of ATP-ase succinic DHG, lactic DHG, G-6-P·DHG, NADH-diaphorase, NADPH-diaphorase, estradiol-DHG and testosteron-DHG in the hepatic cells of type II (Hh) of EIA were stronger than those of other enzymes. Generally the enzyme activities in this type II were lower than those in type I.

c) High activities of alkaline P-ase, acid P-ase, succinic DHG, NADH-

hepatic cells of EIA- and non EIA-horses.

Various enzymes in hepatic cells											
Alkaline phosphatase	Acid phosphatase	ATP-ase	Succinic DHG	Lactic DHG	α-glycero-P. DHG	NAD. DHG	NADP. DHG	Testosteron DHG	Estradiol DHG	Monoamine Oxidase	G.6.P DHG
###	###	###	###	###	+	###	###	++	###	++	###
###	++	++	###	++	++	###	###	###	###	++	++
++	++	++	++	++	++	###	###	###	###	++	###
###	###	++	++	++	++	###	###	###	###	###	###
++	###	###	++	++	++	###	###	###	###	++	###
###	###	###	###	###	++	###	###	###	###	++	###
###	###	++	++	++	++	###	###	###	###	++	###
###	###	++	###	###	###	###	###	###	###	###	###
+	++	###	###	###	++	###	###	++	###	++	###
###	###	###	###	###	+	###	###	###	###	###	###
###	###	++	###	++	++	###	###	###	###	++	++
###	###	++	###	++	+	###	++	++	###	++	++
++	###	###	###	++	+	###	###	###	++	++	++
###	###	###	###	++	+	###	###	###	++	###	###
###	###	###	###	++	+	###	++	###	++	###	++
###	###	###	###	++	+	###	###	###	++	###	###
###	###	###	++	++	+	###	++	###	++	###	++
###	###	++	++	+	+	###	###	###	++	###	+
###	###	###	###	###	++	###	###	###	++	++	++
++	###	++	++	++	+	###	###	###	###	++	###

diaphorase, NADPH-diaphorase, testosteron-DHG, estradiol-DHG in only case of the type III in EIA were indicated.

d) Generally, the activities of alkaline P-ase, acid P-ase, succinic DHG, NADH-diaphorase, and estradiol-DHG were stronger than those of other enzymes in the type IV (hh) of EIA.

e) The observed number of cells with high activities of enzymes in the EIA-hepatic cells arranged in ascending order are i.e. Type HH (numbers of high activities/total observing numbers, 10/12)>Type Hh (8/12)>Type hH(7/12)>Type hh (5/12). Accordingly the strongest activities of enzymes are in the hepatic and splenic hypersiderosis.

f) Hepatic hemosiderosis did not find in the cases of non-EIA: The activities of alkaline P-ase, acid P-ase, ATP-ase, succinic DHG, NADH-diaphorase, NADPH-diaphorase, G-6-P·DHG and testosteron-DHG were stronger than those

Table 2. Distribution of various enzymes in the

Enzyme & Hemosiderosis					Hemosiderosis				
					Liver		Spleen		
*	**	***	****	*****	Hepatic cells	Hepatic RES	Trabecule	Follicles	Sinuses
EIA	I	HH	D	Y 1	⫴	⫴	−	+	⫴
			D	S 1	⫴	⫲	+	⫲	⫲
			N	S 2	⫴	⫴	−	⫴	⫲
			N	S 3	⫴	⫴	+	+	⫲
			N	S 4	⫴	⫴	⫲	⫴	⫴
			N	S 5	⫴	⫴	⫴	⫲	⫴
			N	S 6	⫴	⫴	+	⫲	⫴
			N	S 7	⫲	⫲	−	−	⫲
	II	Hh	D	M 1	⫲	⫲	⫲	−	−
			D	Y 2	⫲	⫲	⫲	−	−
	III	hH	D	Y 3	+	⫲	−	−	⫲
	IV	hh	D	M 2	+	+	−	−	−
non-EIA	III	hH	−	S 87	−	−	⫲	−	⫲
			−	S 89	−	−	+	−	⫲
			−	S 91	−	−	−	−	⫴
			−	S 107	−	−	−	+	⫴
			−	S 168	−	−	⫲	−	⫴
			−	S 190	−	+	−	−	⫴
	IV	hh	−	S 143	−	−	−	−	+
			−	S 189	−	−	−	−	+
			−	S 191	−	−	−	−	+
			−	S 203	−	−	−	−	+

* EIA or non-EIA ** Type *** Type of hepatic and splenic hemosiderosis

Remarks: D, diffuse from of hemosiderosis; N, nodular form of hemosiderosis in the

in the Type hH of non-EIA.

g) In the Type hh of non-EIA there are indications of higher activities of alkaline P-ase, acid P-ase, NADH-diaphorase, NADPH-diaphorase and testosteron-DHG than in those of the others.

h) Generally there were indications of higher activities of various enzymes in the hepatic cells such as the hepatic and splenic hypersiderosis of EIA than those in the ones such as the hepatic and splenic hyposiderosis of non-EIA. Especially interest was the existence of high activities of testosteron-DHG and estradiole-DHG in EIA and those of testosteron-DHG in non-EIA.

(2) *Enzyme-histochemical changes in the hepatic RES of EIA- and non EIA-horses*

The results in the present investigation are shown in Table 2.

a) In the Type HH of EIA with strong hemosiderosis of hepatic RES, the

hepatic RES in the liver of EIA- and non EIA-horses.

Type of hepatic hemosiderosis	Enzymes in hepatic RES											
	Alkaline phosphatase	Acid phosphatase	ATP-ase	Succinic DHG	Lactic DHG	α-glycero-P. DHG	NAD. DHG	NADP. DHG	Testosteron DHG	Estradiol DHG	Monoamine oxidase	G.6.P DHG
D	−	+	+	−	++	+	+	−	+	+	++	+
N	+	++	+	+	+	+	++	++	+	+++	++	+
N	+	+++	+++	+	+++	++	+++	+	+++	−	+	+
N	+	+++	+++	−	++	+++	+++	+	+++	+	−	++
N	+	+++	+++	−	+++	+++	++	+	+++	−	−	++
N	++	+++	+++	−	++	++	++	+	+++	−	−	−
N	+++	++	++	−	+++	+	+++	+	++	−	−	+
D	+++	++	+	−	+++	++	+++	+++	+	+++	++	+
D	++	++	+	++	+++	+	++	+++	++	+++	++	++
D	++	+++	+	++	+++	+	++	++	+	+++	++	+
D	+++	++	+	++	++	++	++	++	++	+++	++	++
D	+	+	+	++	++	+	++	+	++	+++	++	++
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−

**** Type of hepatic siderosis ***** Name of Horses

hepatic RES; M, Miyagi Prefecture, Y. Yamagata Prefecture, S. Shibaura Slaughter House.

activities of acid P-ase, ATP-ase, lactic DHG, NADH-diaphorase and testosteron-DHG were stronger than those of the others. However, in Type HH of EIA without hemosiderosis and with strong lymphoid cell infiltration and activated RES, there were high activities of alkaline P-ase, G-6-P·DHG, lactic DHG, ATP, NADH-diaphorase, NADPH-diaphorase, testosteron-DHG.

b) In the Type Hh of EIA with severe RES-hemosiderosis, the activities of lactic DHG and estradiol-DHG were higher than those of the others. On the other hand, in Type Hh of EIA with asiderosis and lymphoid cell infiltration, high activities of G-6-P·DHG, lactic DHG and estradiole DHG were shown.

c) Generally there were indications of strong activities of estradiol-DHG in the strong RES-hemosiderosis of Type hH of EIA, and those of G-6-P·DHG and estradiol-DHG in Type hH of EIA without RES-hemosiderosis.

d) In the RES of EIA with and without hemosiderosis, there were high

Table 3. Distribution of enzymes in the spleens of

*	**	***	****	Alkaline phosphatase: Endth. T.A.	Alkaline phosphatase: Reticulum	Alkaline phosphatase: Sinus-Hd.	Acid phosphatase: Endth. of Sinus.	Acid phosphatase: T. Con. Tis	Acid phosphatase: Sinus-Hd.
EIA	I	D	Y 1	+	++	−	+++	−	−
		D	S 1	+++	++	− −	−	+	−
		N	S 2	−	+++	++	+++	−	−
		N	S 3	++	+	+	−	−	−
		N	S 4	++	+++	+++	+++	+	++
		N	S 5	++	−	−	++	−	++
		N	S 6	−	++	−	+++	−	+++
		N	S 7	−	−	−	++	−	−
	II	D	M 1	+++	−	−	+	−	−
		D	Y 2	+++	+++	−	++	−	−
	III	D	Y 3	+++	+++	++	+++	− −	−
	IV	D	M 2	−	−	−	+	−	−
non-EIA	III	−	S 87	+	++	++	+++	++	−
		−	S 89	−	++	−	++	++	−
		−	S 91	−	+	−	++	−	−
		−	S 107	−	+++	−	+++	−	++
		−	S 168	+	−	−	+	−	−
		−	S 190	−	++	−	+++	+	−
	IV	−	S 143	+	++	−	+++	+	+
		−	S 189	−	++	−	+	−	−
		−	S 191	−	++	−	+	−	−
		−	S 203	−	−	−	+s	−	−

* EIA or non-EIA ** Type *** Type of hepatic siderosis **** Name

Remarks: Endth., endothelium; T., trabecular; Hd., hemosiderosis;

activities of G-6-P·DHG and estradiole-DHG in the Type hh of EIA.

e) There were no activities of enzymes in the Types hH and hh of non-EIA.

(3) *Enzyme-histochemical changes in the spleen of EIA- and non EIA-horses*

The results on the distribution of splenic enzymes are shown in Table 3.

a) The activity of alkaline P-ase in the endothelium of the trabecular artery was high in type HH and Hh of EIA, but low in Type hH and hh of EIA.

b) Acid P-ase appeared weakly in the connective tissue of the trabecule, but strongly in the endothelium of splenic sinusoids. There were no indications of differences among the various types.

c) There were no activities of α-glycerophosphate DHG in the spleen.

d) The activitity of G-6-P·DHG were strongly positive in the endothelium of blood vessels in the trabecule, but weakly positive in the connective tissue of the trabecule.

EIA- and non-EIA horses.

α-glycero-P. DHG	G-6-P. DHG				NADPH. DHG (TPNH)		NADH. DHG (DPNH)	MAO		
Total spleen	T. Con. Tis.	T. artery	Pencil A.	Sheath A.	T. Con. Tis.	Endth. T.A.	Total spleen non-EIA	T. Con. Tis.	Pencil A.	Sheath A.
−	++	+++	++	+++	−	+++	+++	+	+	+
−	+	++	+++	+++	++	++	+++	−	−	−
−	+	+++	+++	+++	+	++	+++	+	+	−
−	+	+++	++	++	+s	+++	+++	+++	−	−
−	+s	−	−	++	−	+++	+++	+	+	−
−	++	−	++	++	−	−	+++	−	−	−
−	−	+++	++	++	−	−	+++	++	+	−
−	−	+++	+++	+++	−	−	+++	+	+	−
−	+	+++	+++	+++	−	++	+++	−	−	−
−	+	+++	+++	+++	−	−	+++	+	−	−
−	−	−	+++	+++	−	−	+++	+	−	−
−	+	+++	+++	+++	+	−	+++	−	−	−
−	−	+++	+++	+++	−	+++	+++	−	+	+
−	−	++	++	++	−	++	+++	−	−	−
−	−	−	+++	+++	−	+++	+++	++	−	+
−	−	++	+++	+++	−	+++	+++	+	++	−
−	−	++	+++	+++	−	++	+++	−	+	−
−	−	+	++	++	−	−	+++	−	−	−
−	−	++	++	++	−	+++	+++	+	−	−
−	−	++	++	++	−	−	+++	−	+s	−
−	++	−	+++	+++	−	+	+++	+++	−	−
−	−	++	+++	+++	−	−	++	−	−	−

Con. Tis., connective tissue; A., artery

e) Generally the activity of NADH-diaphorase was strongly positive, and the activity of NADPH-diaphorase was strong in the endothelium of the trabecular artery.

f) The activity of MAO was irregular.

Summary and Conclusion

Studying 12 enzymes in the liver and 7 enzymes in the spleen; there were investigations on the enzyme-histochemical changes in 8 Type HH, 2 Type Hh, 1 Type hH and 1 Type hh of EIA, and 6 Type hH and 4 Type hh of non EIA. Fig. 1 indicate the distribution of the high activities of various enzymes in the liver and spleen. Observing portions of the liver and spleen were divided into hepatic lesions (1. hemosiderosis in the hepatic cells, 2. hemosiderosis in the capillary endothelium and Gliesson's sheath, 3. activation of RES and lymphoid cell infiltration in the capillary wall and Gliesson's sheath without hemosiderosis, and 4. hepatic cells without hemosiderosis) and splenic lesions (1. connective tissue of the trabecule, 2. trabecular artery, 3. arterial capillaries in the follicle, 4. intrafollicular reticulum, 5. endothelium on the pencil artery and sheath artery, and 6. endothelium of sinuses). The numeral character in the above-described hepatic and splenic lesions indicated the structural position in Fig. 1. The abbreviations in the Fig. 1 indicate the following: Alk., alkaline P-ase; Acid., acid P-ase; ATP., adenosine triophosphatase; SDHG, succinic dehydrogenase; LDHG, lactic dehydrogenase; NADH, NADH-diaphorase; NADPH, NADPH-diaphorase; Testosteron., testosterone-DHG; Estradiol., estradiol-DHG; MAO, monoamine oxidase; and G-6-P, G-6-P dehydrogenase.

Of special interest was the appearance of high activities of enzymes in the liver and spleens of Type HH, Hh, hH and hh from the points of the interrelationship between hepatic and splenic hemosiderosis.

1) EIA-lesions of Type HH and Hh with severe hepatic hypersiderosis had higher activities of the enzymes than that of Type hH and hh with hepatic hyposiderosis. These changes were similar to non EIA-lesions of Type hH and hh with hepatic hyposiderosis. These changes were similar to non EIA-lesions of Type hH and hh. Non EIA-lesions indicated hepatic asiderosis-splenic hypersiderosis Type hH and hepatic asiderosis-splenic asiderosis in Type hh.

2) EIA seemed to be devided into two groups the high activities of enzymes in Type HH and Hh, and the low activities of enzymes in Type hH and hh. Accordingly two different forms might be thought to exist an active form in the former types and a dormant form in the latter types.

3) Furthermore, there existed a good-balance of high enzymatic activities of testosteron-DHG and estradiol-DHG in Type HH of EIA; inbalance of enzymatic activities estradiol-DHG>testosteron-DHG in Type Hh, hH and hh of EIA; and no estradiol-DHG in both Type hH and hh of non-EIA. From this point it might

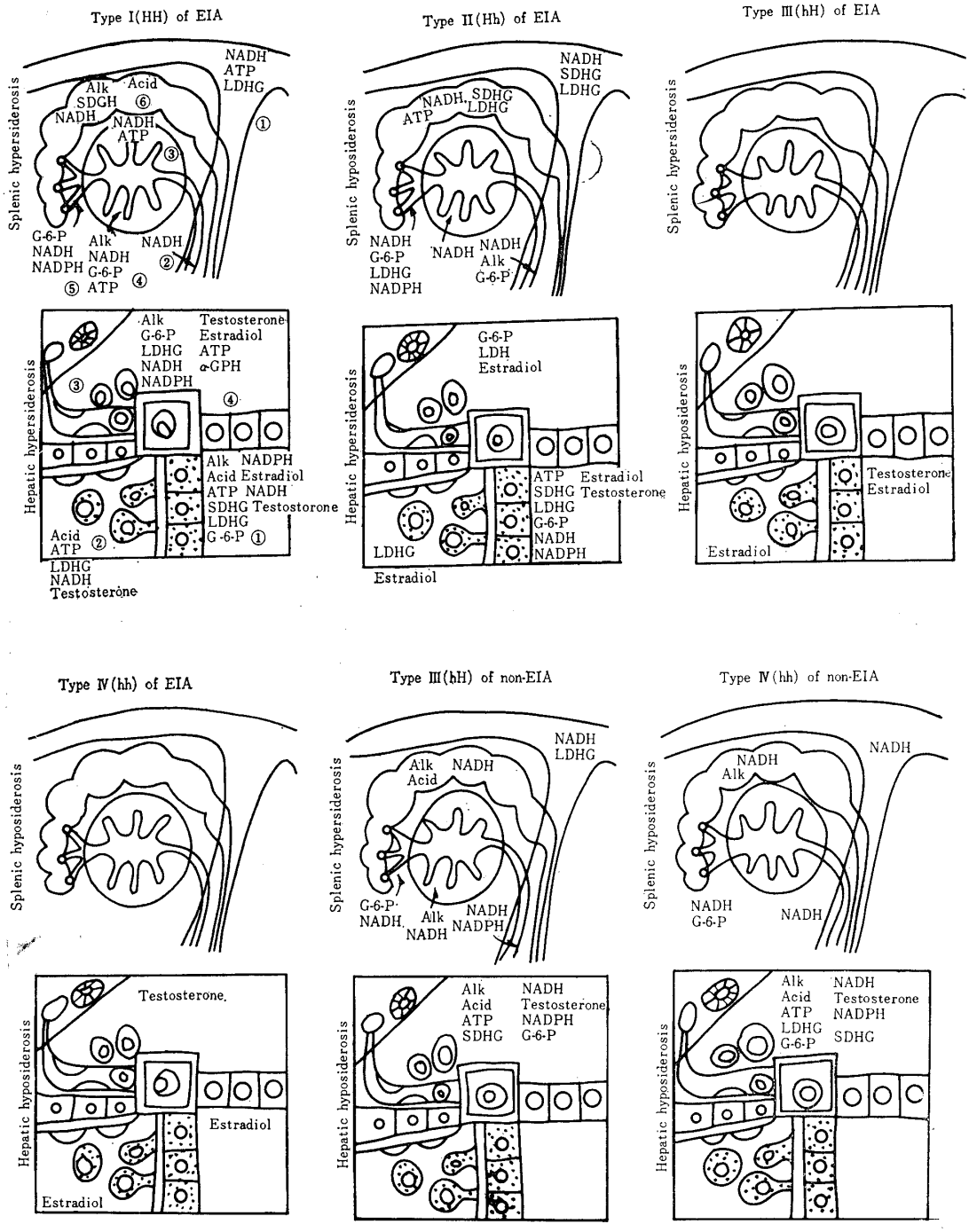

Fig. 1. Schematic figures indicated the distribution of various enzymes with high activities in the livers and spleens of Type I (HH), II (Hh), III (hH) and IV (hh) of EIA-and non EIA-horses.

be important to find the role of testosteron-DHG and estradiol-DHG as the factor to control Type HH, Hh, hH and hh.

To discuss the enzyme-histochemical results investigated in EIA and non EIA, it would be important to see the biochemistry of erythrocytic metabolism (21).

Observing the relationships between hepatic-splenic hemosiderosis in EIA and non EIA, and enzymatic activities in the liver and spleen, the enzymatic activities in the liver and spleen, the enzymatic activities in Type HH and Hh of EIA were similar to that in Type hH and hh of non EIA. In this point it was clear that the activities of G-6-P DHG, NADH-diaphorase, NADPH-diaphorase, ATPase, and lactic DHG related to the erythrocytic metaoblism depressed in the Type hH and hh of EIA. Accordingly it might be important in future to investigate whether the former Type HH and Hh would be chronic or the latter Type hH and hh to be acute.

Moreover it is very important to solve enzyme-histochemically the problems of G-6-P DHG, NADH-diaphorase, NADPH-diaphorase and lactic DHG of the erythrocytes, siderocytes, blood-histiocytes in the peripheral blood of horses affected with EIA virus. This will become the subject for future investigation.

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Plate 1.

Fig. 1. High activity of alkaline phosphatase in the liver of EIA-horse (Yamagata No. 2).

Fig. 2. High activity of alkaline phosphatase in the liver of non EIA-horse (Shibaura No. 91).

Fig. 3. High activity of acid phosphatase in the liver of EIA-horse (Shibaura No. 6).

Fig. 4. High activity of acid phosphatase in the liver of non-EIA-horse (Shibaura No. 91).

Fig. 5. High activity of adenosin tri phosphatase in the liver of EIA-horse (Miyagi-No. 1).

Fig. 6. High activity of adenosin tri phosphatase in the liver of non EIA-horse (Shibaura-No. 191).

Fig. 7. High activity of succinic dehydrogenase in the liver of EIA-horse (Yamagata No. 2).

Fig. 8. High activity of succime dehydrogenase in the liver of non EIA-horse (Shibaura No. 191).

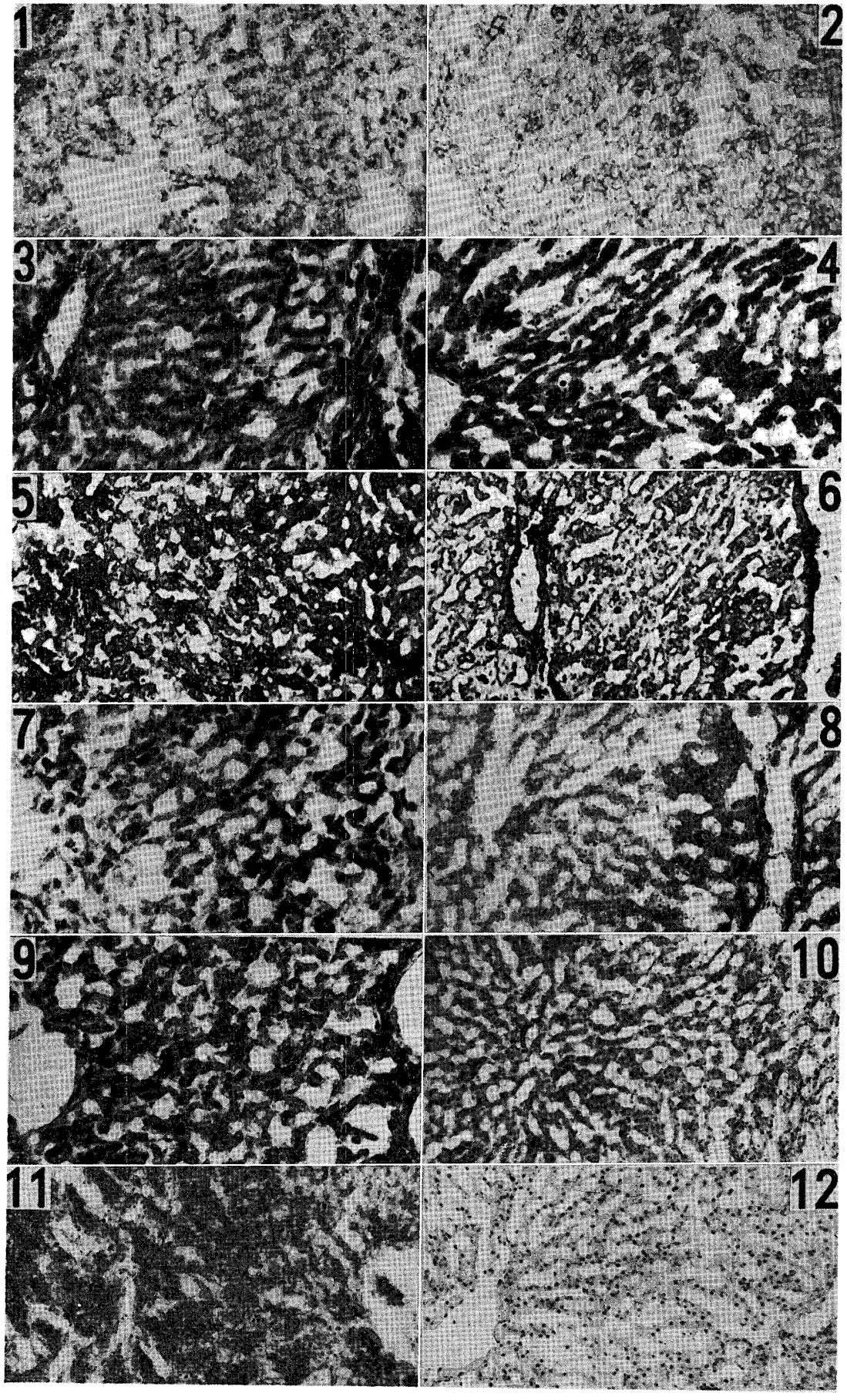
Fig. 9 High activtiy of lactic dehydrogenase in the liver of EIA-horse (Yamagara No. 2).

Fig 10. Low activity of lactic delydrogenase in the liver of non EIA-horse (Shibaura No. 191).

Fig. 11. High activity of α-glycerophosphate-dehydrogenase in the liver of EIA-horse (Shibaura No. 7).

Fig. 12. Low activity of α-glycersphosphate-dehydrogenase in the liver of non EIA-horse (Shibaura No. 203).

All photos were taken at the enlargement of 100×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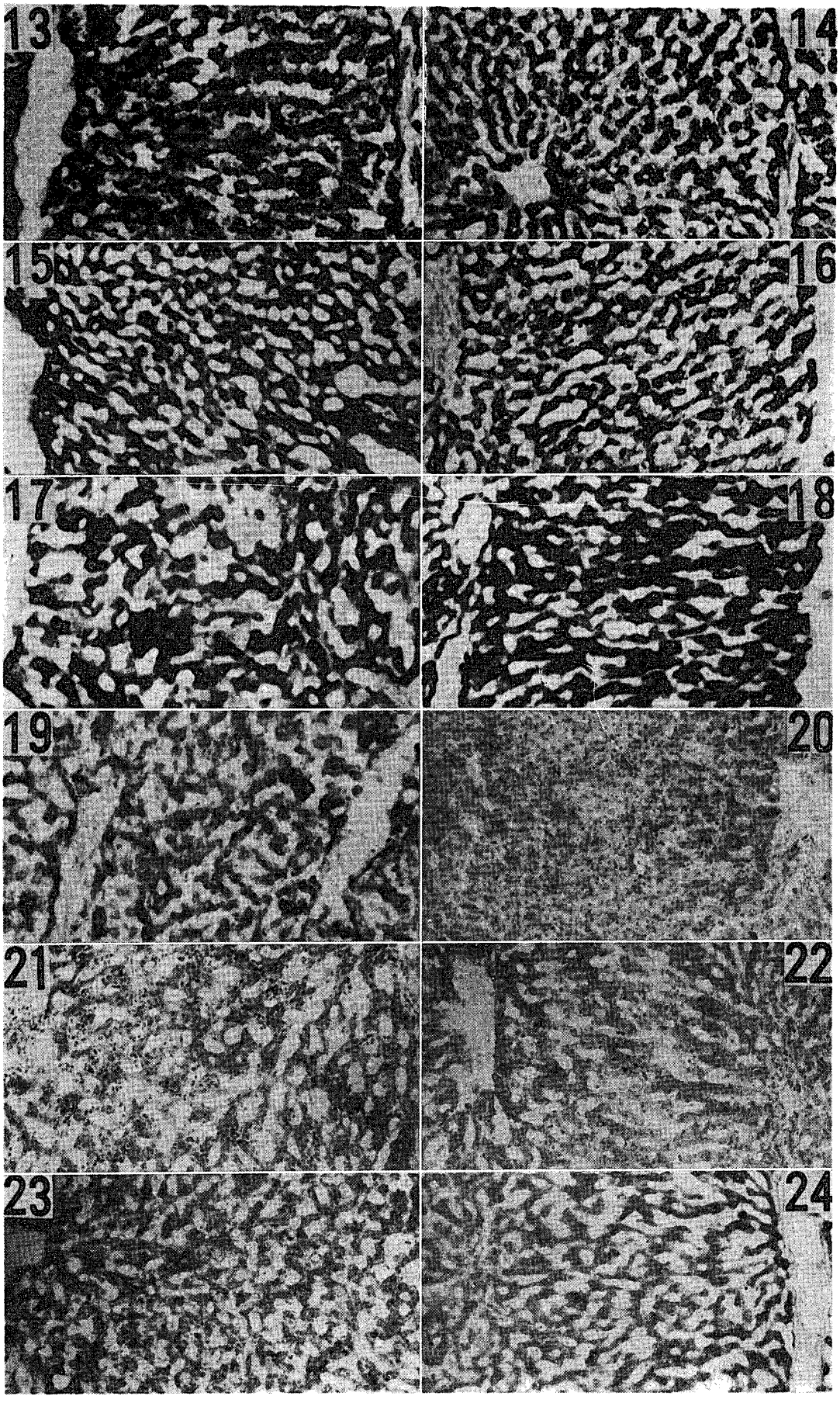
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Plate 2.

Fig. 13. High activity of NAD-diaphorase in the EIA-horse (Yamagata No. 2).

Fig. 14. High activity of NAD-diaphorase in the non EIA-horse (Shibaura No. 191).

Fig. 15. High activity of NADPH-diaphorase in the EIA-horse (Yamagara No. 2).

Fig. 16. High activity of NADPH-diaphorase in the non EIA-horse (Shibaura No. 191).

Fig. 17. Low activity of testosteron-dehydrogenase in the liver of EIA-horse (Miyagi No. 1).

Fig. 18. High activity of testosteron-dehydrogenase in the liver of non-EIA-horse (Schibaura No. 191).

Fig. 19. High activity of estradiol-dehydrogenase in the liver of EIA-horse (Shibaura No. 7).

Fig. 20. Low actioity of estradiol-dehydrogenase in the liver of non EIA-horse (Shibaura No. 87).

Fig. 21. Low activity of monoamine-oxidase in the liver of EIA-horse (Miyagi No. 1).

Fig. 22. Low activity of monomamine-oxidase in the liver of non EIA-horse (Shibaura No. 203).

Fig. 23. High activity of glucose-6-phosphate dehydrogenase of EIA-horse (Yamagata No. 2).

Fig. 24. High activity of G-6-P dehydrogenase of non EIA-horse (Shibaura No. 203).

Plate 3.

Fig. 25. High activity of acid phosphatase in nest-like siderose of the liver of EIA-horse (Shibaura No. 4).

Fig. 26. High activity of adenosine triphosphatase in the nest-like siderosis of the liver of EIA-horse (Shibaura No. 5).

Fig. 27. High activity of lactic dehydrogenase in the nest-like siderosis of the liver of EIA-horse (Shibaura No. 4).

Fig. 28. Low activity of G-6-P dehydrogenease in the nest-like siderosis of the liver of EIA-horse (Shibaura No. 4).

Fig. 29. Low activity of NADP-diaphorase in the nest-like siderosis of EIA-horse (Shibaura No. 1).

Fig. 30. High activity of NAD-diaphorase in the nest-like siderosis of EIA-horse (Shibaura No. 4).

Fig. 31. High activity of NAD-diaphorase in the intime and media of the trabecular artery of EIA-horse (Shibaura No. 4).

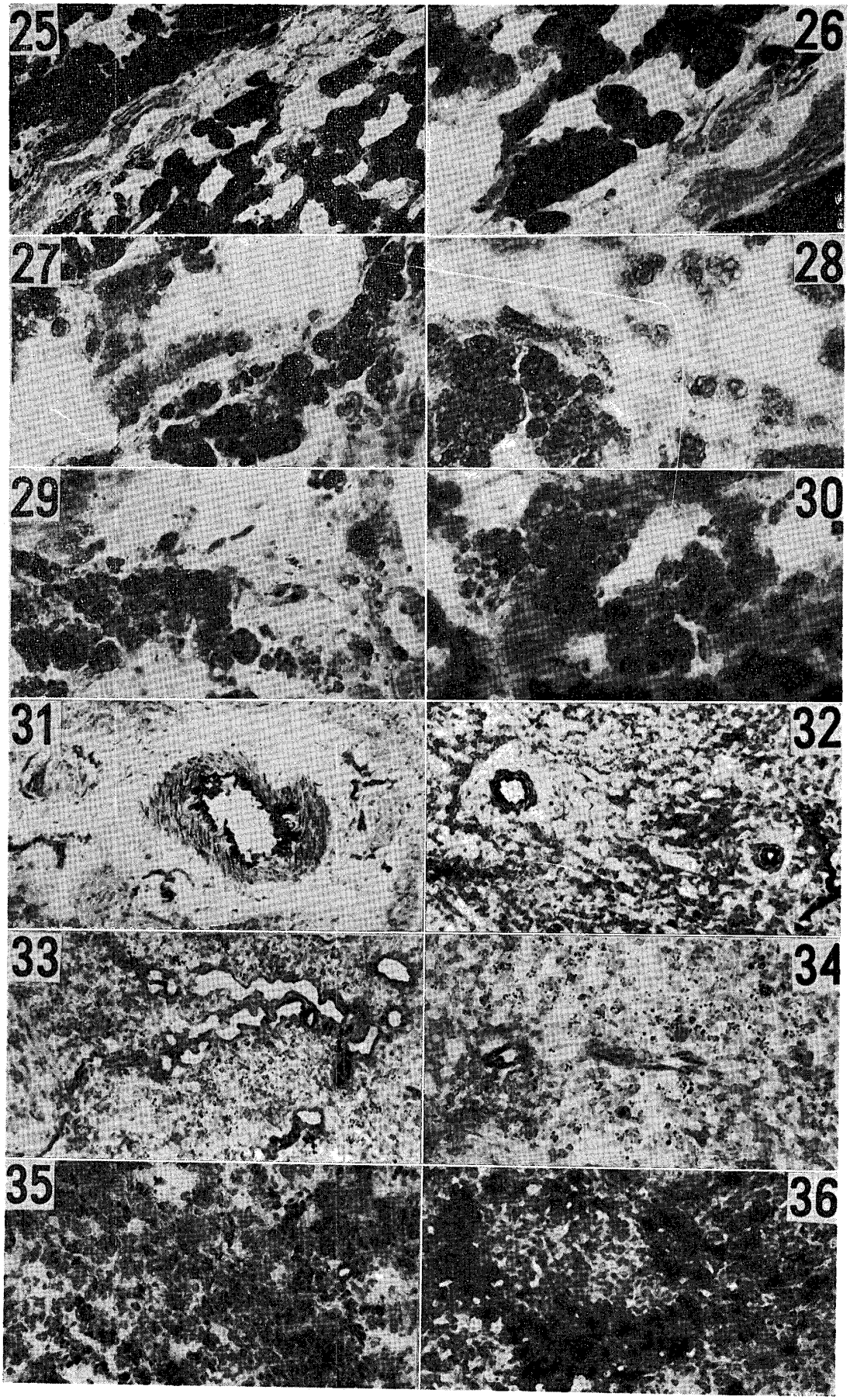
Fig. 32. High activity of NAD-diaphorase in the intime of the central artery of EIA-horse (Shibaura No. 4).

Fig. 33. High activity of NAD-diaphorase in the endothelium of the pensil-artery of EIA-horse (Shibaura No. 4).

Fig. 34. High activity of NAD-diaphorase in the of EIA-horse (Shibaura No. 4).

Fig. 35. High activity of acid-phosphatase in the splenic sinuses of EIA-horse (Shibaura No. 4).

Fig. 36. High activity of alkaline phosphatase in the reticulum cells of EIA-horse (Shibaura No. 4).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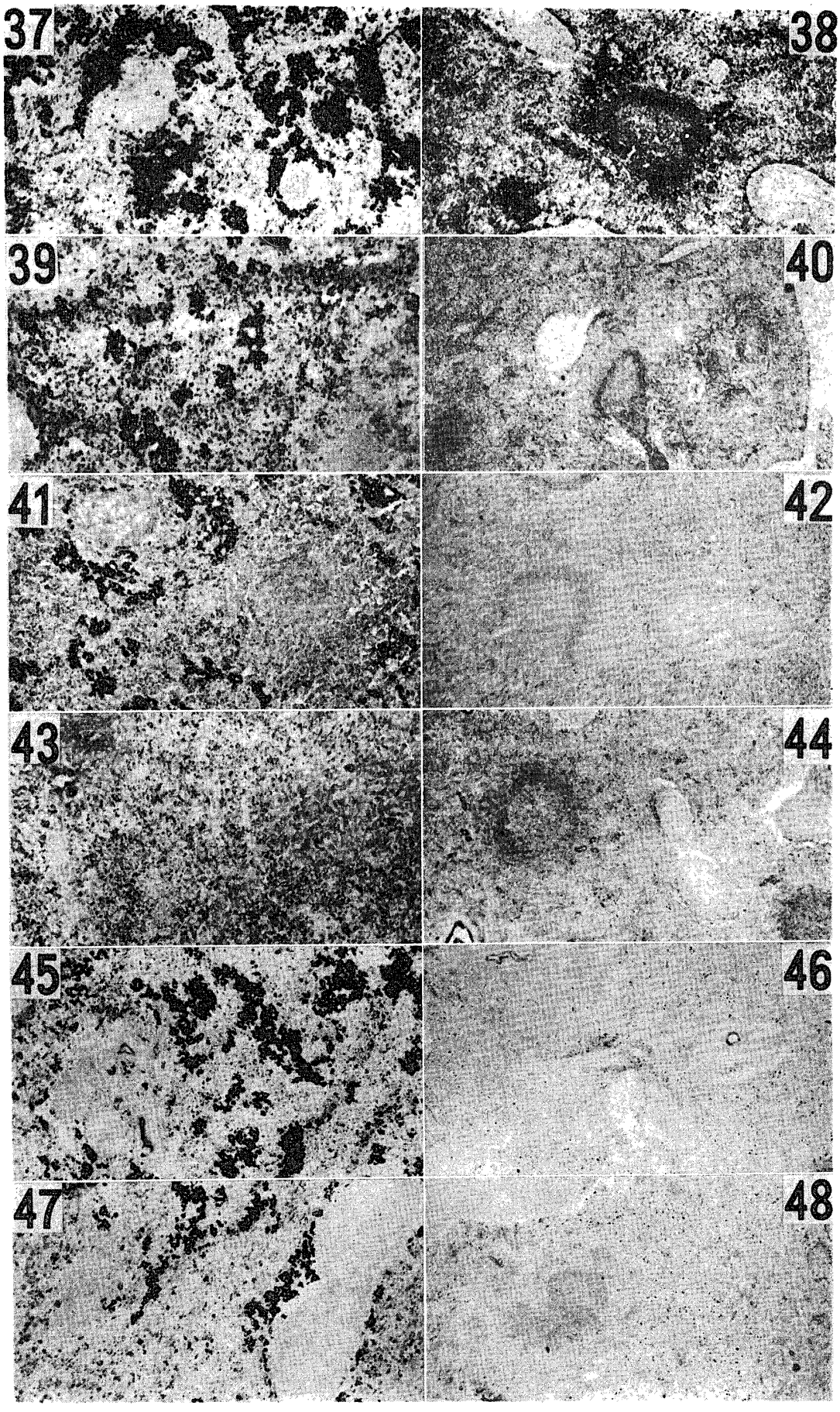
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Plate 4.

Fig. 37. High activity of alkaline phosphatase in the spleen of EIA-horse (Shibaura No. 4).

Fig. 38. Low activity of alkaline phosphatase in the spleen of non EIA-horse (Shibaura No. 191).

Fig. 39. High activty of acid phosphatase in the spleen of EIA-horse (Shibaura No. 4).

Fig. 40. Low activity of acid phosphatase in the spleen of non EIA-horse (Shibaura No. 191).

Fig. 41. High activity of adenosine-triphosphtase in the spleen of EIA-horse (Shibaura No. 4).

Fig. 42. Low activty of adenosine-triphosphatase in the spleen of non EIA-horse (Shibaura, No. 191).

Fig. 43. High activity of NAD-diaphorase in the spleen of EIA-horse (Shibaura No. 7).

Fig. 44. Low activity of NAD-diaphorase in the spleen of non EIA-horse (Shibaura No. 191).

Fig. 45. High activity of NADP-diaphorase in the spleen of EIA-horse (Shibaura No. 4).

Fig. 46. Low actioity of NADP-diaphorase in the spleen of non EIA-horse (Shibaura No. 191).

Fig. 47. High activity of G-6-P dehydrogenase in the spleen of EIA-horse (Shibaura No. 4).

Fig. 48. Low activity of G-6-P dehydrogenase in the spleen of non EIA-hourse (Shibaura No. 191).