Chapter I

INTRODUCTION

Metaplasia of bone marrow and the behavior of the reticulo-endothelial system have been most usually studied in the pathology of equine infectious anemia (EIA) during past half century.

But also it should be noted that pathological studies have recently been performed in Japan on the actual situation of the disease (YAMAGIWA and co-workers [13~17]).

Interest has been aroused in the clinical observations on the disease, especially in hematological tests (ISHII and co-workers[4~6]) and biopsy of visceral organs. Methods of liver puncture were studied and discussed with considerable results by YASUDA et al. (1941), MIURA and WADA (1943), SUGANO et al. (1950), and ICHIKAWA et al. (1955).

Biopsy has also been applied to the spleen. This seems to institute a new approach to the clinical observation of the disease. Spleen puncture was first used in cytological studies by MIURA (1952), who attached much importance to the behavior of monocytes, plasma cells, and granulocytes, especially that of monocytes. SAKAI and TAMURA (1953) described an increase of lymphocytes of small size revealed by spleen puncture in a naturally infected case of chronic type.

It is keenly felt that studies on spleen punctate should be carried out with renewed efforts in individual cases of different types and stages, a wide observation on the whole body being taken into consideration with special reference to the findings in peripheral blood, liver-puncture tissue, and bone-marrow punctate.

Adopting this point of view, the author has been making detailed studies of cells of spleen punctate harvested from naturally and experimentally infected cases of different stages as well as control cases.

Although more precise cytological studies are to be performed in the future, the characteristics of spleen puncture as an ante-mortem diagnostic means are reported in the present paper, which it is hoped may contribute to the advancement of the histopathology of the disease.

This work has been carried out at the Hokkaido Branch of the National Institute of Animal Health, by courtesy of Dr. R. IRIE, Chief of the Branch. The present

author wishes to thank Professor S. YAMAGIWA, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Hokkaido University, for his indispensable supervision and advice throughout this work. Gratitude is expressed to Dr. M. KOBAYASHI, the former Director of the Institute, and to Dr. S. ISHII, the actual Director, for the facilities made available and for permission to publish this work. The author is indebted to the staff of the Livestock Section of Hokkaido Prefectural Government, for the supply of natural cases of EIA. Thanks are also due to Mr. M. NAKAYAMA and Miss T. TAKAHASHI for their technical assistance and to Mr. M. ITO for preparing the photomicrographs.

Chapter II

MATERIALS AND METHODS

Materials

Normal group Specimens of spleen punctate were collected from 20 horses one to six times each, totaling 49. Those of bone-marrow punctate were harvested from 11 horses. All the horses were adults of 4 to 15 years of age, except one which was 2 years old.

Immunized group This group consisted of hyperimmunized horses kept at the Hokkaido Branch of the National Institute of Animal Health for the production of equine paratyphoid serum. Specimens of spleen punctate were collected from 15 horses, to a total number of 24, and those of bone-marrow punctate from 8 horses.

Infectious anemia group The natural cases numbered 16, all of which had been diagnosed definitely as EIA. Ten of them were sacrificed, immediately after puncture, at the slaughterhouse or farms on which they were kept, to harvest specimens for histopathological examination. The remaining six were kept at the stable of the laboratory for chronological observation of the course of the disease. Four cases were subjected to vital staining with 5 per cent lithium carmine solution.

Nine horses were used as experimental cases. During an observation period of several days before inoculation of viral material, it was ascertained in these horses that there were no abnormalities in body temperature nor in the findings in peripheral blood, liver-puncture tissue, and spleen punctate. All the animals, except one, were sacrificed 13, 14, 22, 22, 23, 32, 41 and 113 days, respectively, after inoculation. The remaining one succumbed 397 days after inoculation.

The viral materials used were jugular-vein blood in 10 per cent sodium citrate (9 : 1), sternal bone-marrow punctate in 10 per cent sodium citrate (1 : 9), and organ emulsion. The emulsion, after mixed with 3 parts of physiological saline solution, was centrifuged at 3,000 rpm for 10 minutes and the resulting supernatant was used for inoculation.

Methods

Spleen puncture The puncture needle (Figures 1 & 2) was a pointed, sheathed steel needle 7.0 cm in length, 0.2 cm in outside diameter, and 0.1 cm in

inside diameter. The site of puncture was selected on a parallel line passing through the tuber coxae at the anterior border of the left 18th rib of a horse held in the right position. The harvested sample was sucked up in a 10-ml injection cylinder and blown out on a clean slide glass. Erythrocytes and leucocytes were counted and smears prepared. In general, 0.3 ml was sucked up for erythrocytes and 0.5 ml for leucocytes. Smears were subjected to the May-Giemsa staining, Berlin blue reaction, and peroxydase reaction (copper sulfate method and ARMITAGE method). Some of them were subjected to supravital staining with Janus green and neutral red and used for microscopy by means of phase-contrast apparatus. The differential count of nucleated cells is expressed in percentages by counting 500 to 1,000 cells in the smear stained with the May-Giemsa stain.

Bone-marrow puncture Sternal bone-marrow puncture was performed with the same needle as used for spleen puncture. The sample collected by puncture was treated according to the method described for spleen puncture.

Liver puncture A commercial puncture needle was employed. The site of puncture was chosen generally between the 14th and 15th ribs on the right side. The harvested liver tissue was fixed with formalin, embedded in paraffin, cut thin, and stained with hematoxylin and eosin for microscopic examination.

Peripheral blood examination Blood samples were taken from the jugular vein for erythrocyte and leucocyte counts, erythrocyte sedimentation rate, hemochromometry, siderocyte detection (ISHII's method), and differential leucocyte count on smears stained with the Giemsa stain.

Post-mortem and histopathological examination Post-mortem dissection was conducted on 25 horses of the EIA group. Tissue samples were collected from them, fixed with formalin, embedded in paraffin, and stained with hematoxylin and eosin and with some special stains, if necessary, for microscopic examination.

Chapter III

RESULTS OF OBSERVATIONS

A Various Phases of Cells, Especially Basophil Round Cells, in Spleen Punctate (Text figure 1, Plates II & III)

Erythrocytes: Erythroblasts and nucleated erythrocytes are not regular components of the spleen. They were not observed in any case of EIA.

Lymphocytes: Lymphoblasts are of large size and have well-defined, slightly basophilic cytoplasm. The nucleus is spherical or slightly flattened spherical and contains 2 or 3 nucleoli and dense, fine chromatin network. There is no indentation in the nucleus. Lymphocytes of large size have well-defined cytoplasm which is generally clear with no outstanding characteristics, but which sometimes contains azure granules and vacuoles. These cells seldom contain a nucleolus. In fresh specimens, the nuclei are spherical or ovoid and sometimes have an indentation. Chromatin network is loose in most nuclei. Lymphocytes of small size are not different from those present in the peripheral blood. They have restricted cytoplasm which sometimes contains azure granules and is basophilic. Their nuclei are small and spherical and often have an indentation. Chromatin network is fine and very dense. In supravital staining specimens, mitochondria are observed, like those of other animals, as short rod-like structures stained with Janus green and agglomerating mostly on one side.

Monocytes: Monocytes have well-defined cytoplasm; they are generally weakly basophilic, but sometimes strongly so. The nucleus is irregular or flattened spherical in shape with roughness. The chromatin network is coarse and woven with well-balanced distribution of light and dark portions. Some of them have one or two nucleoli. Such cells are of juvenile form. The cells of mature form all have lobated (from 2- to 4-lobed) nuclei with chromatin network which is characteristic for its loose but regular arrangement. (Chromatin network is so arranged in the cells of both juvenile and mature forms as to look like to facade of a large building with many windows.) Some of the cells have granules while others contain vacuoles and foreign bodies in cytoplasm. Supravital staining well reveals the character of monocytes, in which fine, homogeneous particles of neutral red have a tendency to form a rosette or to agglomerate in the indented portion

EXPLANATION OF TEXT FIGURE 1

Supravital staining (neutral red and Janus green) of cells contained in spleen punctate

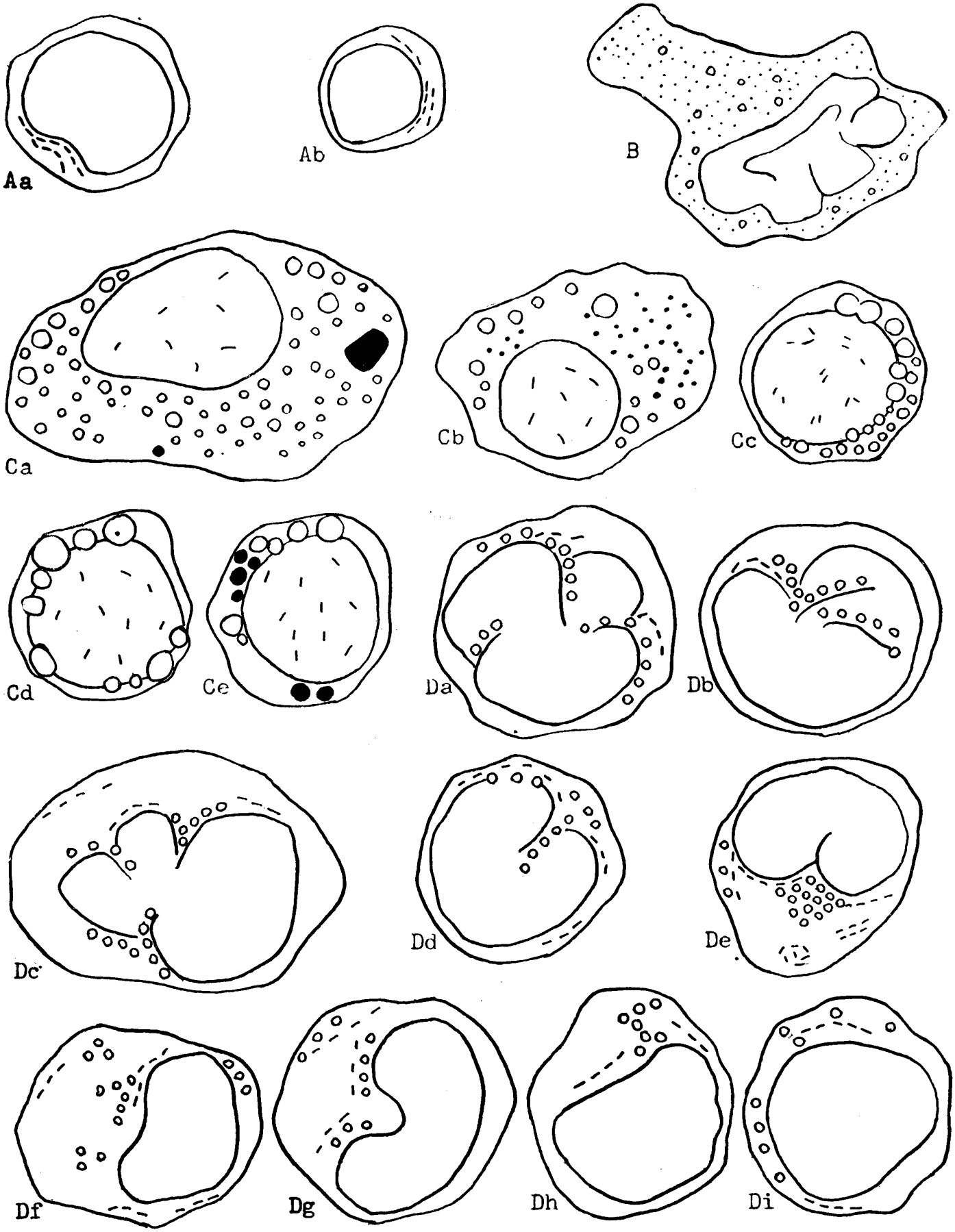
A: lymphocytes—short rods in the cytoplasm are mitochondria stained with Janus green.
  Aa: cell with one depression
  Ab: cell with no depression

B: a neutrophil leucocyte—black spots in the cytoplasm are neutrophilic granules showing Brownian movement. Small circles are neutral-red granules.

C: reticulum cells
  Ca & b: reticulum cells with wide cytoplasm (macrophages) Many black spots, small and large, are foreign bodies phagocytized. Small circles in the cytoplasm are neutral-red granules of irregular size.
  Cc, d & e: reticulum cells with narrow cytoplasm (round-transformed cells). Neutral-red granules fill the cytoplasm around the nucleus. Black spots are foreign bodies phagocytized. The nucleus is round and smooth.

D: monocytes—small circles are neutral-red granules. Short rods are mitochondria stained with Janus green. The neutral-red granules of the cytoplasm are of almost the same size in the monocytes.
  Da, b & c: cells with clearly lobulated nucleus; neutral-red granules are arranged along the indentation of the nucleus.
  Dd, e, f & g: cells with less conspicuous indentation; there is a tendency for neutral-red granules to gather at the notch or depression.
  Dh & i: cells with obscure notch or depression. In these cells, the demarcation of the nucleus is not smooth, mitochondria are present, and neutral-red granules are fine, being different from those in reticulum cells.

TEXT FIG. 1.



of the nucleus. Mitochondria stainable with Janus green are short rods; they are observed aggregating in a clump or two. When examined by the phase-contrast microscope, cells which have such character all show depression, indentation, or lobulation of the nucleus. In diameter they are 1.5 to 2 times as large as erythrocytes. A great majority of them give negative and few of them weakly positive for peroxydase reaction.

Various cells of reticulo-endothelial system: Typical reticulum cells have well-defined, homogeneous, bright cytoplasm of a blue color; they often contain 1 or 2 small foreign bodies. The nucleus is ellipsoid in general but polymorphous in rare cases; it always has loose, fine chromatin network and 1 or 2 nucleoli. Supravital staining reveals smooth, spherical or ellipsoid nuclei and wide cytoplasm which often has projecting pseudopods and granules of neutral red of different sizes scattered diffusely. It is originated from cells of such kind that macrophages are formed.

Round-transformed cells have a narrow cytoplasm, which contains erythrocytes or Berlin-blue reaction positive, coarse granular foreign bodies in some cells (siderocytes); the cytoplasm in others is light brown or gray in color. The nucleus of round-transformed cell is regular spherical, situated on one side, and has a somewhat coarse network instead of the typical reticular structure; in some cells there is a nucleolus. These cells are regular components of normal spleen punctate. In supravital staining, the nucleus is smooth and regular spherical; the cytoplasm is narrow and filled with neutral red which forms small brilliant granules of various sizes and encircles the nucleus like the corona around the sun. Some cells contain black spot-like foreign bodies. No mitochondria stainable with Janus green are seen.

Endothelial cells have ellipsoid nuclei with loose, fine chromatin network; these cells have spindle-shaped or long belt-like, bright cytoplasm on both sides of the nucleus. They are sometimes seen in a clump of 2 or 3 cells.

All those cells which are mentioned above are regularly observed as reticulo-endothelial cells in normal spleen punctate. The cells of reticulo-endothelial system are, however, more polyphase in the case of EIA. In the spleen punctate of the disease, the cytoplasm is well-defined with no tinctorial characteristic; the cytoplasm often includes vacuoles and foreign bodies. The nucleus is located in the center or periphery of a cell and is spherical or somewhat flattened spherical, resembling a tennis racket. Chromatin network is fine and relatively dense. Most cells have no nucleoli. The principal part in the reticulo-endothelial cell reaction observed in the early stage of EIA is played mostly by cells of such kind as described above, or by a form of round-transformed cells. Mononuclear or polymorphonuclear cells of macrophage form are seldom present in normal

spleen punctate, but are easily found in specimens collected in the chronic to the relapsing stages of EIA.

Various cells of granulocyte origin: Most of the neutrophil leucocytes have segmented nuclei and rarely rod-shaped ones. No metamyelocytes and no cells less mature than those are observed at all. Likewise, no juvenile form is found in eosinophil leucocytes and basophil leucocytes. There are no particular findings in the cases of EIA. Peroxydase reaction is positive both in neutrophil leucocyte and eosinophil leucocyte. In supravital staining, neutrophil leucocytes are seen to contain minute granules of neutral red which begin to show Brownian movement in about 60 minutes. Later, fluid vacuolar granules are formed in the cells. Eosinophil leucocytes have brilliant coarse granules of neutral red as well as acidophilic granules. Basophil leucocytes are also filled with small, a little less brilliant granules of neutral red as well as basophilic granules.

Rupture of nucleus: Among the cells, some contain a nucleus broken up into small pieces in the cytoplasm (karyorrhexis), some have an irregular-shaped nucleus shrunk, condensed, and stained more deeply than normal and homogeneous in the dark cytoplasm (pyknosis), and others contain 2 or 3 brown, Berlin blue reaction positive granules in the shady cytoplasm with the nucleus disappearing. Although the rupture or breaking up of the cell nucleus is not often found, such change is almost always seen in such cases. Besides, there are some other cells with very irregular-shaped nuclei which have connection with some form of the rupture or breaking up of the cell nucleus (unidentified cells).

Remarks on basophil round cells

"Basophil round cell" is a designation by which the most important morphological characteristic is stressed, and it has no connection with any conventional name similar to it. Of course, it has a connection with neither basophil granulocyte, nor monocyte with basophilic cytoplasm.

The term "basophil round cells" is used for convenience' sake only when the composition of cells in spleen punctate is studied quantitatively. It has only a just comprehensive meaning.

"Basophil round cells" include two kinds of cells, λ cells and plasma cells. Morphologically, λ cells have well-defined, wide cytoplasm and are strongly basophilic uniformly. They sometimes have a localized unstained area near the nucleus or in some other portion of the cytoplasm. It is very seldom that a vacuole is contained in them. The nucleus is spherical and centrally-located and has no depression, indentation, nor lobulation. Chromatin network is fine regularly woven and somewhat dense. It is completely different from that of

plasma cells and of small-sized lymphocytes; it is also easily distinguishable from that of cells of the reticulo-endothelial system. Such λ cells rather resemble erythroblasts in early stage, but they are not so flat-shaped as the latter and are easily distinguishable when compared. Pictures of mitosis and amitosis are often shown in them. Large-sized cells have one or two nucleoli. No phagocytic function is recognized in λ cells.

Plasma cells have a bright area on one side of the nucleus or in the wide, strongly basophilic cytoplasm. The chromatin network of plasma cell gives an impression of being woven firmly with thick trabeculae showing wheel-like arrangement.

In the normal spleen punctate, "basophil round cells" are low in ratio and consist mostly of plasma cells there being few λ cells.

Quite differently, λ cells occupy an important part of the findings in spleen punctate and plasma cells play very little role in the case of EIA.

Lambda cells resemble plasma cells in appearance more than any other cell does, but they are different from the latter in characters. It may easily be assumed that the tissue from which they have originated is the reticulo-endothelial system, but their characters do not always justify this assumption. The mother tissue of these cells is found in such an organ which has lymphoid tissue as the spleen or the lymph nodes. Bone marrow is an organ in which exist myeloid and reticulum tissues and a less amount of lymphoid tissue. When investigation is performed on the spleen, an organ in which lymphoid tissue is prevalent, bone marrow is useful as an interesting control. In the examination of bone marrow conducted parallel to that of the spleen, neither λ cells nor plasma cells show original reaction nor do they play any important role at all in bone marrow.

The period of λ-cell reaction is limited to the early stage of infection. As the disease takes a chronic course, the site of the reaction is shifted to other cells. No tendency is observed for the reaction to appear again when a relapse of the disease takes place.

Referring the papers published on EIA, the author can cite the "L" cells of YAMAGIWA et al. (1952) as being similar in description to those mentioned above. Although there must be some difference in the definition between the "L" cells and the λ cells described above, the two forms may be identical cytologically and histologically.

Next, the term "lymphoid cell" has often been used since a long time ago.

The view of DOBBERSTEIN (1934) on this cell type is as follows: "Some investigators regard the lymphoid cell as bone-marrow element or embryonic blood cell for the interpretation of myelometaplasia. Others consider it as degenerated histiocyte or cell emigrating from the spleen or the bone marrow. COHRS deems

it as reticulo-endothelial cell differentiated to produce antibody.··· The animal body responds to the virus of EIA with histiocyte reaction, lymphoid-cell reaction, and fibrillar reaction. Histiocyte reaction is remarkable at the early stage and lymphoid-cell reaction becomes clear with the advancement in days of the disease".

Those cells which form the important basis of the myelometaplasia theory appearing in the relatively early stage of investigation, may be in the same category with the λ cells mentioned above. It is not worth while discussing whether the λ cells have relations with myeloid tissue.

More investigation is necessary for lymphoid cells. MIURA (1952) first described monocyte reaction in the spleen punctate of EIA. The author also has observed it. Nothing definite has been proved as yet about the mother tissue of monocytes and the course of monocyte reaction.

Those cells similar to lymphocytes which show reaction in the spleen and the lymph nodes in the chronic stage of EIA and which the author calls lymphoid cells in the sense of DOBBERSTEIN, have not been distinguished as yet from lymphocytes of small size by any of the workers on spleen punctate, including SAKAI and TAMURA (1953) and the present author. It should be noted that YAMAGIWA and his associates (1952)[15] described "the increase of lymphocytes and static activity of the reticulo-endothelial system" and the presence of two types of cell reaction in chronic types in their histopathological studies.

Above is a summary of the literature on λ cells. For years, until the theory of "L" cells was published, the λ cell had supplied one of the bases of the myelometaplasia theory. It was also a rather well-known fact that some investigators had regarded the λ cell as belonging to the reticulo-endothelial system whilst others had generally included it among the lymphoid cells.

B Findings in Spleen Punctate and Bone-marrow Punctate of Normal and Immune-serum-producing Horses (Tables 1 & 2)

It is superfluous to give all the numerical data on normal spleen punctate; they were subjected to statistical treatment to obtain the confidence limit of mean value and rejection limit by the transformation of percentage to degrees. At the same time, examination was made on the presence of significant differences between the data obtained from the splenic pulp bloods and those from the splenic sinus bloods (at the level of 5 per cent).

It is necessary to differentiate between pulp blood and sinus blood in the spleen punctate harvested. This can be demonstrated statistically and from experience.

The erythrocyte count is higher in splenic blood than in peripheral blood. This fact indicates that the spleen is an organ to hold erythrocytes physiologically

TABLE 1. *Splenograms of Normal and Immunized Horses*

	CONFIDENCE LIMIT OF POPULATION MEAN (95%)			
	Normal (49 cases)		Immunized (24 cases)	
	Pulp	Sinus	Pulp	Sinus
Erythrocytes (10^6/mm^3)	10.84±0.97	8.73±1.83	9.05±1.63	
Nucleated cells (10^3/mm^3)	32.7 ±6.2	16.0 ±4.3	22.9 ±5.9	
Lymphatic cells	%	%	%	%
Lymphoblasts	0.05±0.01		0.04±0.02	
Large lymphocytes	17.54±0.21	6.38±0.32	13.87±0.27	5.00±0.50
Small lymphocytes	49.67±0.10	31.77±0.40	43.08±0.12	31.01±0.15
Total	70.28±0.15	38.95±0.15	58.03±0.21	37.76±0.69
Basophil round cells	0.92±0.03	0.30±0.07	0.73±0.03	0.60±0.02
Monocytes				
Juvenile form	1.90±0.03	1.73±0.05	1.93±0.13	2.40±1.15
Mature form	2.09±0.02	2.84±0.14	3.03±0.03	4.64±0.12
Total	4.13±0.05	4.61±0.16	5.30±0.12	7.61±0.62
Reticulo-endothelial system				
Reticulum cells	0.75±0.02	0.38±0.03	0.63±0.02	0.47±0.16
Round-transformed cells	0.78±0.02	0.36±0.13	0.85±0.02	0.58±0.07
Siderocytes	1.55±0.03	0.53±0.12	0.76±0.04	0.34±0.38
Endothelial cells	0.04±0.01	0.02±0.02	0.04±0.02	0.01±0.04
Total	3.68±0.03	1.60±0.13	2.70±0.03	1.70±0.10
Neutrophil leucocytes	18.63±0.15	49.23±0.15	26.85±0.26	47.25±0.43
Eosinophil leucocytes	1.11±0.02	2.30±0.11	1.77±0.01	1.82±1.62
Basophil leucocytes	0.15±0.01	0.53±0.07	0.22±0.02	0.16±0.12
Unidentified cells	0.01±0.01	0.04±0.06	0.14±0.04	0.24±0.59
Karyorrhexis	0.67±0.03	0.42±0.27	0.09±0.04	0.13±0.54

and suggests that the spleen is not the mother tissue of cells of the erythrocyte group. Nucleated-cell count is much higher in spleen punctate than in peripheral blood and shows significant difference between pulp blood and sinus blood. In the splenic pulp, most nucleated cells are cells of the lymphocyte group. "Basophil round cells" are mostly composed of plasma cells and constitute a constant ingredient, though their ratio of occurrence is low. Monocytes are divided into two forms, mononuclear and segmented-nuclear, corresponding to the juvenile and mature forms, respectively. They are more abundant in the sinus blood than in

TABLE 2. *Myelograms of Normal and Immunized Horses*

	CONFIDENCE LIMIT OF POPULATION MEAN (95%)	
	Normal (11 cases)	Immunized (8 cases)
Erythrocytes (10^6/mm^3)	5.68± 0.74	6.40± 1.69
Nucleated cells (10^3/mm^3)	141.0 ±54.0	147.0 ±73.0
Cells in process of Granulopoiesis	%	%
Myeloblasts	0.49±0.05	0.26±0.12
Promyelocytes	0.75±0.16	
Neutrophil myelocytes	1.06±0.14	1.02±0.07
Eosinophil myelocytes	0.39±0.15	0.06±0.05
Basophil myelocytes	rare	rare
Metamyelocytes	27.0±0.23	2.80±0.13
Rod-shaped cells	17.04±0.21	11.80±0.20
Segmented cells	14.90±0.60	18.20±0.44
Eosinophil leucocytes	1.66±0.11	1.50±0.18
Basophil leucocytes	0.12±0.06	0.18±0.06
Total	41.95±0.26	37.51±0.76
Megakaryocytes	+	+
Basophil round cells	1.05±0.08	0.38±0.12
Lymphocytes	6.37±0.52	5.72±0.73
Reticulum cells	0.88±0.10	0.52±0.05
Monocytes		
Juvenile form	1.84±0.08	0.65±0.04
Mature form	1.18±0.12	0.62±0.03
Total	3.20±0.11	1.32±0.02
Cells in process of Erythropoiesis		
Urerythroblasts	0.78±0.15	0.49±0.03
Erythroblasts in early stage	3.26±0.28	2.54±0.18
Erythroblasts in late stage	20.76±0.23	25.07±0.24
Normoblasts	17.49±0.22	24.08±0.37
Total	43.48±0.47	52.90±0.99
Karyorrhexis	0.13±0.06	
Mitosis	0.66±0.04	0.85±0.04

the pulp, because some of them have originated from blood and also they seem to flow out rapidly from the pulp. The quantity of monocytes ranks third next to neutrophil leucocytes. Monocyte reaction is observed to occur especially in immune-serum-producing horses. Next, a picture of erythrocyte destruction is shown by round-transformed cells (siderocytes) belonging to the reticulo-endothelial system. The proportion of these cells is low in immune-serum-producing horses. Granulocytes are abundant in sinus blood; they consist mostly of neutrophil leucocytes. No presence of mother tissue is demonstrable, but there is a tendency for neutrophil leucocytes to increase in immune-serum-producing horses.

The data on bone-marrow punctate were studied statistically as were those of spleen punctate. The erythrocyte count is almost the same in the bone marrow as in the peripheral blood, but the nucleated-cell count is much higher. Nucleated cells comprise mostly cells of the granulocyte and the erythroblast groups. The number of erythroblasts in bone marrow is much larger in horses than in man. Megakaryocytes are absorbed with difficulty. "Basophil round cells" are composed mostly of plasma cells. Both forms, juvenile and mature, are observed among the monocyte components. Lymphocytes are small in number and contain no other cells than those of peripheral blood origin. No remarkable difference is observed between immune-serum-producing horses and normal ones.

The degree of mixture with peripheral blood is roughly estimated in the spleen punctate by the number of neutrophil leucocytes and in that of bone-marrow punctate by the number of lymphocytes.

C Observations on Natural Cases of Equine Infectious Anemia

1 Relationship between findings in spleen punctate and pathological changes of spleen tissues

The findings in spleen punctate are believed to show the characteristics of pathological changes of spleen tissues. To what extent can parallel relationship between findings in punctate and tissue be observed? To solve this problem, such relationship was studied on horses, the spleen punctate of which was obtained by ante-mortem puncture and which were sacrificed immediately (within 2 to 3 hours) after puncture.

Among the pathological changes of spleen tissue, particularly noteworthy cell reactions are those of reticulo-endothelial cells, lymphoid cells (in the sense used by DOBBERSTEIN)* and λ cells. The findings in spleen punctate, when observed from this point of view, were divided into two, those in which λ-cell reaction was outstanding and those in which it was not. Specimens from cases Nos. 1～6 belonged to the former group and Nos. 7～10 to the latter group.

* Hereinafter referred to as lymphoidc ells simply.

The λ cell is very characteristic either in tissue specimen or spleen punctate specimen. It is not difficult at all to find a close relationship between the two.

In general, it is difficult to find any characteristic pathological changes in the bone-marrow punctate. Those cases which showed spleen punctate with λ-cell reaction are mostly anemic in peripheral blood. In those cases with λ-cell reaction, the pathological change of the liver was one of the systemic changes and mostly included remarkable activation of cells of the reticulo-endothelial system. Livers with no remarkable λ-cell reaction presented a nodular form, in which multiple nodules were formed in the lobules and filled with lymphoid cells.

2 Continual observations on findings in spleen punctate, liver tissue, peripheral blood and bone-marrow punctate

In the preceding section (C–1) it was stated that findings in spleen punctate were divided into two, those with λ-cell reaction and those without it, and that the former reflected the pathological changes of spleen to some extent. However, it is necessary to study various pathological changes further from different angles in order to establish the significance of λ-cell reaction in EIA. For this reason, 6 natural cases of EIA were investigated.

Report of cases

Case 1, subacute type, HAKUAI by name, female, adult

In November, 1951, when clinical examination was performed, no remarkable changes were found in clinical conditions and siderocyte test was negative. It was on April 28, 1952, that the patient lost activity and appetite and ran high fever, giving positive siderocyte test. From that date, this patient was observed continually as a case of EIA.

The period of observation was 20 days from April 28 to May 17, 1952. It ran continued fever and revealed moderate anemia. The spleen punctate presented almost constantly λ-cell, monocyte, and reticulo-endothelial-cell reactions. The liver tissue obtained by puncture showed of small-sized foci of necrobiosis. Post-mortem histopathological changes included λ-cell reaction in the spleen and the lymph nodes, activation of cells of the reticulo-endothelial system in the whole body, and degeneration of parenchyma. Besides, vital staining was performed just before destruction by injecting 5 per cent lithium carmine solution daily in 9 doses totaling 2,300 ml.

Case 2, subacute type, No. 11, female, adult

During working in animal farm N for tilling, this horse ran fever and revealed anemia. On November 2, 1950, liver puncture was performed and the specimen suggested of EIA. The animal was observed continually from March 28, 1951.

The observation period was 43 days from March 28 to May 9, 1951. The case ran recurrent fever twice. Anemia was moderate. The λ-cell, monocyte, and reticulo-endothelial-cell reactions were observed almost constantly in the spleen punctate. In the specimen of liver tissue by puncture, sloughing cells were scattered in the sinusoids. Post-mortem histopathological examination revealed λ-cell reaction in the spleen and the lymph nodes. The histopathological findings of the liver were the same at biopsy and autopsy. This patient succumbed during vital staining after the third injection; a total amount of 1,000 ml of stain, was given to it.

Case 3, relapsing type, No. 12, female, adult

This mare was diagnosed as a true case of EIA in the periodical examination by the staff of the Livestock Section of Hokkaido Prefecture. On the same day she gave a normal birth.

The observation period was 27 days from May 5 to May 31, 1951. She ran continued fever. Anemia was of high degree. During this observation period she was nursing her colt. As emaciation and weakness increased gradually, she fell at last into astasia and was sacrificed after having been subjected to vital staining (7 doses totaling 1,700 ml daily).

The spleen punctate showed almost constantly monocyte and reticulo-endothelial-cell reactions. The liver tissue by puncture presented a nodular form filled with lymphoid cells. Post-mortem histopathological changes included activation of cells of the reticulo-endothelial system in the whole body and degeneration of parenchyma.

Case 4, chronic type, No. 2, female, adult

This mare was diagnosed as a true case of EIA in the periodical examination by the staff of the Livestock Section of Hokkaido Prefecture.

Observation continued for 368 days from January 27, 1950, to January 29, 1951. The patient showed recurrent fever 7 times. Although no abnormality was observed in erythrocyte count at an early stage, the anemic condition was gradually revealed in the count. A slight monocyte reaction was shown in the spleen punctate at the later stage of the observation period (at 298 & 321 days). The lesion of liver tissue by puncture was of a nodular form. Lymphoid-cell reaction in the spleen and the lymph nodes was observed in post-mortem histopathological sections. The same pathological change as at biopsy was to be seen in the liver at autopsy.

Case 5, chronic type, SHIKISHIMA by name, female, adult

She was pregnant, but abortion was caused by the administration of *Salmonella abortivoequina*. The results of liver puncture performed on the day of abortion

indicated that she was a case of EIA.

From that time she was held under continual observation for a period of 72 days from February 13 to April 25, 1951. Recurrent fever was manifested 3 times. Anemia was not remarkable. Monocyte reaction was found in the spleen punctate. The lesion of liver tissue by puncture showed a nodular form. Post-mortem histopathological examination revealed lymphoid-cell reaction in the spleen and the lymph nodes. Also, vital staining (8 doses totaling 3,300 ml daily) was carried out just prior to destruction.

Case 6, chronic type (association), No. 19, female, adult

This animal was diagnosed as EIA in the periodical examination conducted by the staff of the Livestock Section of Hokkaido Prefecture.

The observation period was 134 days from June 24 to November 4, 1952. She showed a tendency of remittent fever and anemia. An abscess on the shoulder became worse and erupted by itself, discharging pus, from which *S. abortivoequina* was isolated. A gradual increase in emaciation and debility caused astasis, which preceded death. The cause of death was secondary septicemia developed from pyothorax.

Monocyte reaction was observed in the spleen punctate. The lesion of liver tissue by puncture was of a nodular form. In the examination of bone-marrow punctate, rod-shaped cells and metamyelocytes were found to be increasing. Post-mortem histopathological examination revealed lymphoid-cell reaction in the spleen and the lymph nodes, a slight activation of cells of the reticulo-endothelial system, and hyperemia, hemorrhage, and bacterial embolism of various organs.

Summary

In those cases which were considered to be of subacute type from their ante- and post-mortem findings, λ-cell, monocyte, and reticulo-endothelial-cell reactions were almost constantly observed in the spleen punctate for a certain period of time. In the cases of chronic type, hyperplasia of lymphoid cells was recognized as a histopathological change. These lymphoid cells, however, were not observed as particular characteristic cells in the spleen punctate and most of them were regarded as lymphocytes of small-size. The cases of relapsing type could not be identified particularly before death, although activation of cells of the reticulo-endothelial system was remarkable as a histopathological change in them.

APPENDIX on Vital Staining

Vital staining with lithium carmine was performed on five horses, including one which will be described in the following section. Only one horse, No. 11, died during the staining, manifesting shock-like symptoms immediately after

injection.

The progress of staining in the liver could be observed by puncture. Stellate cells of KUPFFER, endothelial cells of capillaries, and hepatic cells were the first to show phagocytic function after a very small amount of stain had been injected. Hepatic cells made a beautiful appearance with fine carmine granules scattered over the cytoplasm. In horse No. 12 injected with the lithium carmine of 150 ml (the carmine of 7.5 g), stellate cells of KUPFFER and endothelial cells were positive and hepatic cells negative. In the horse called BOTAN injected with 550 ml (27.5 g), hepatic cells were positive to a traceable extent. Among the cells which reacted, histiocytic cells and macrophages were positive, but lymphoid cells and small, round sloughing cells showed no phagocytic function in the nodules, sinusoids, and GLISSON's capsules. In the spleen, although positive reaction was given by endothelial cells of the sinuses and connective-tissue cells of the capsule and trabeculae, it was considerably difficult to turn pulp cells positive. Among the cells which did react, reticulum cells forming foci of round-transformed cells and proliferation foci were positive. The λ cell and lymphoid cell showed no phagocytic function at all. The finding to the stain of the lymph nodes resembled that of the spleen. The most outstanding phagocytic function was played by endothelial cells of the sinus in the lymph nodes. In the case of sinus catarrh, most cells in the lymph sinus were positive to the stain. Among the cells reacting in lymphoid tissue, reticulum cells forming foci of round-transformed cells and proliferation foci were positive, but λ cells and lymphoid cells were all negative. In the kidneys, epithelial cells of the convoluted uriniferous tubules manifested intake function as well as the hepatic cells. Epithelial cells of the collective tube had a weaker intake function than those of the convoluted uriniferous tubules. As was observed in horse No. 12, those epithelial cells which underwent degeneration while the animal was living could not exert phagocytic function and, accordingly, were very clearly distinguishable from the healthy ones. Among the cells which reacted, histiocytic cells were positive, but lymphoid cells aggregating in the interstitial tissue were almost all negative. Capillary endothelial cells of the adrenal cortex also revealed phagocytic function, though they were inferior in this activity to the corresponding cells of the liver and sinus endothelial cells of the spleen and the lymph nodes. In the lungs, positive cells were scattered only in capillaries of the alveolar wall; vascular endothelial cells themselves showed no phagocytic function. Among the cells aggregating in the interstitial tissue of the myocardium, histiocytic cells displayed phagocytic function, but other cells were almost all negative.

Positive cells were not always contained in those cells which were absorbed into spleen punctate.

D Observations on Experimentally Infected Cases of Equine Infectious Anemia

1 Cases under long-term observation (Tables 3 & 4)

Report of cases

Case 1, No. 9, female, 9 to 10 years of age

Observation was started on August 1, 1950. On October 30, 80 ml of sodium-citrated blood of a naturally infected case were inoculated by the subcutaneous route. The first appearance of pyrexia was recorded on the 26th day of the disease.* Thereafter recurrent fever appeared 14 times before death. Anemia progressed gradually and emaciation became remarkable. At last astasis was caused and death occurred on the 397th day of the disease.

Findings in spleen punctate The first puncture was carried out on the 41st day of the disease. The λ-cell, reticulo-endothelial-cell, and slight monocyte reactions were observed on that day. All these reactions were shown also on the 124th day of the disease, but the count of λ cells was lower and that of monocytes higher than those obtained from the preceding examination. After that, spleen puncture was performed 23 times up to the 393rd day of the disease. Although monocyte reaction was observed constantly, λ-cell and reticulo-endothelial-cell reactions disappeared completely. The nucleated-cell count showed increasing and erythrocyte count decreasing trend.

Findings in bone-marrow punctate The bone marrow absorbed by puncture was examined after the 242nd day of the disease. Erythrocyte count tended to show gradual decrease. The erythroblast group showed also gradually decreasing formation. In the granulocyte group, rod-shaped and segmented cells were increasing. Monocyte reaction was also observed.

Findings in peripheral blood Erythrocyte and leucocyte counts decreased remarkably at the time of the first pyrexia. After that, with the progress of the disease, erythrocyte count recovered temporarily and then became decreased, but leucocyte count was increasing. Increasing counts were shown in neutrophil leucocytes in the febrile stage and in lymphocytes in the non-febrile stage. The erythrocyte sedimentation rate was high in the febrile stage and always high in the final stage of the course of the disease. Siderocytes were observed for the first time on the 41st day of the disease. They were detected in the largest quantity in the course of the disease when it had progressed to chronic. Anemia of high degree and neutrophilia were manifested immediately before death.

Findings in liver tissue Nothing particular was noted in the liver tissue obtained by puncture on the 27th, 35th and 41st days of the disease. On the 63rd

* The first day of the disease is the day when viral material was inoculated.

TABLE 3. *Splenogram of Horse No. 9*

	DAYS AFTER INOCULATION																								
	41	124	140	154	170	184	207	220	234	242	248	257	264	271	277	284	294	300	314	321	356	363	371	378	393
Erythrocytes (10^6/mm^3)				9.84	5.65		9.29	10.15	8.42	9.96	9.33	7.28	9.76	8.70	8.25	7.79	3.81	5.96		7.01	4.99	3.37	3.37		
Nucleated cells (10^3/mm^3)			42.2	27.0	17.2	78.4	45.0	56.7	70.6	44.8	35.2	34.4	60.8	44.8	70.0	48.2	76.2	54.2	47.6	48.4	62.6	36.2	32.0		
Lymphatic cells	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Lymphoblasts	0	0.2	0	0.2	0	0	0.2	0	0.4	0.4	0.2	0.2	0.4	0.2	0	0	0	0.2	0	0.4	0.6	0.2	0.2	0	0
Large lymphocytes	13.2	4.4	6.0	3.6	8.6	3.2	4.8	7.8	5.6	5.8	4.2	5.4	4.6	4.0	4.2	0.2	11.0	2.4	3.6	5.0	6.4	9.4	8.4	6.0	7.6
Small lymphocytes	40.0	45.8	44.0	31.4	38.4	63.0	55.2	44.6	67.0	47.6	64.6	66.2	65.4	45.8	50.8	61.4	66.8	54.4	60.6	67.4	71.4	58.0	50.0	60.0	62.6
Total	53.2	50.4	50.0	35.2	47.0	66.2	60.2	52.4	73.0	53.8	69.0	71.8	70.4	50.0	55.0	61.6	77.8	57.0	64.2	72.8	78.4	67.6	58.6	66.0	70.2
Basophil round cells	8.2	3.0	1.4	1.0	0.6	0.8	1.2	0.8	2.2	1.0	1.8	1.6	0.8	1.4	1.4	1.8	0.8	0.8	0.4	0.4	0.8	1.2	0.8	0.6	2.6
Monocytes																									
Juvenile form	5.4	11.2	8.8	13.6	9.0	5.8	14.6	6.6	7.8	12.4	8.8	7.2	9.2	10.4	17.0	9.4	9.6	10.8	11.8	8.8	5.4	5.4	9.0	7.4	7.4
Mature form	3.4	6.6	6.6	9.6	5.8	5.4	2.0	4.8	4.4	6.2	2.6	5.4	4.6	9.2	5.2	4.8	3.2	5.4	4.4	5.2	3.6	4.4	5.4	4.8	6.2
Total	8.8	17.8	15.4	23.2	14.8	11.2	16.6	11.4	12.2	18.6	11.4	12.6	13.8	19.6	22.2	14.2	12.8	16.2	16.2	14.0	9.0	9.8	14.4	12.2	13.6
Reticulo-endothelial system																									
Reticulum cells	6.4	6.4	4.8	1.8	3.6	1.6	3.6	1.6	2.0	0.8	1.6	1.2	0.6	0.2	1.0	0.2	1.0	0.2	0.2	0.2	0.2	1.4	0.8	0.2	1.4
Round-transformed cells	0.4	3.8	0.8	0.4	1.4	1.0	0.6	0.2	2.6	0.8	3.4	0.8	1.6	0.6	1.4	1.8	0.4	0.8	2.8	1.2	1.4	1.8	0.6	1.4	3.0
Siderocytes	0.2	0.2	0	0	0	0	0.6	0	0.2	0.2	0	0	0	0.4	0	0	0	0	0.4	0	0	0	0	0	0
Endothelial cells	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0.2	0	0.2	0	0	0.2	0.4	0	0	0
Total	7.0	10.4	5.6	2.2	5.0	2.6	5.0	1.8	4.8	1.8	5.0	2.0	2.2	1.2	2.4	2.2	1.4	1.2	3.4	1.4	1.8	3.6	1.4	1.6	4.4
Neutrophil leucocytes	22.0	17.0	22.0	32.0	31.0	15.4	11.4	32.4	5.0	19.8	10.4	11.4	11.0	23.4	16.0	17.8	6.2	19.2	12.0	10.2	9.0	14.6	20.0	16.8	7.8
Eosinophil leucocytes	0.4	1.0	0.4	0	1.0	0	0.4	0.4	0.2	1.2	0.4	0.4	0.4	1.0	0.4	1.0	0.8	2.6	1.0	0.4	0.2	0.6	1.0	1.4	0.4
Basophil leucocytes	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.2	0.2	0.4	0.2	0	0.2	0.2	0.2	0.2	0.6	0.4	0.2	0.4	0.2	0.4	0	0.2
Unidentified cells	0	0	4.8	5.0	0.2	2.4	2.8	0.4	1.0	1.6	1.0	0	1.2	2.2	1.8	1.0	0	0.6	2.0	0.4	0.2	1.2	2.6	1.4	0
Karyorrhexis	0	0	0	1.0	0	1.0	2.2	0	1.4	1.8	0.2	0	0.2	1.0	0.6	0.2	0	1.8	0.4	0.2	0.2	1.0	0.6	0	0.8
Mitosis	0	0	0	0	0	0	0	0	0	0.2	0.4	0	0	0	0	0	0	0	0	0	0	0.2	0.2	0	0

TABLE 4. *Splenogram of* BOTAN

	BEFORE INOCULATION	DAYS AFTER INOCULATION								
		16	30	58	79	92	97	104	106	112	113
Erythrocytes (10^6/mm^3)			13.48	10.68	14.06	6.92	11.82	14.61	9.32	11.02	10.37
Nucleated cells (10^3/mm^3)		25.3	46.1	66.4	25.2	22.4	59.2	33.5	56.5	38.2	30.9
Lymphatic cells	%	%	%	%	%	%	%	%	%	%	%
Lymphoblasts	0.8	0.8	1.0	0.6	0.4	0.4	0.4	0.6	0.8	1.0	1.4
Large lymphocytes	8.0	15.6	19.4	17.6	17.6	13.0	16.4	5.2	4.6	7.0	8.2
Small lymphocytes	22.2	36.8	35.4	51.8	40.2	37.8	44.8	40.2	43.4	45.6	39.0
Total	31.0	53.2	55.8	70.0	58.2	51.2	61.6	46.0	48.8	53.6	48.6
Basophil round cells	1.2	0	4.4	5.0	4.4	3.8	2.8	1.8	0.2	4.2	2.2
Monocytes											
Juvenile form	4.4	3.0	8.0	5.6	10.0	6.0	2.4	8.8	12.2	9.6	10.8
Mature form	3.0	4.2	3.4	1.8	3.4	4.8	3.2	9.2	9.0	6.0	5.4
Total	7.4	7.2	11.4	7.4	13.4	10.8	5.6	18.0	21.2	15.6	16.2
Reticulo-endothelial system											
Reticulum cells	0.4	0.6	0.6	0.6	2.4	1.0	0.2	1.4	0.6	0.4	0.6
Round-transformed cells	1.0	1.6	6.6	1.4	4.2	1.2	2.0	2.8	4.6	4.2	2.8
Siderocytes	0	2.0	2.6	2.6	1.0	0.2	0	1.6	0.4	1.2	0.2
Endothelial cells	0.2	0	0	0	0	0	0	0	0.2	0	0
Total	1.6	4.2	9.8	4.6	7.6	2.4	2.2	5.8	5.8	5.8	3.6
Neutrophil leucocytes	55.2	32.2	15.0	11.2	14.6	30.6	26.0	25.4	22.2	19.4	28.0
Eosinophil leucocytes	2.4	2.2	1.0	1.2	0.6	0.6	1.6	2.4	0.6	0.8	0
Basophil leucocytes	0.6	0	0.6	0.6	0.2	0.2	0.2	0	0.4	0.4	0.6
Unidentified cells	0.6	0.8	1.6	0	1.0	0.4	0	0.4	0.2	0	0.6
Karyorrhexis	0	0.2	0.4	0	0	0	0	0.2	0.6	0	0.2
Mitosis	0	0	0	0	0	0	0	0	0	0.2	0

day, endothelial cells swelled and increased in number, and sloughing of a small number of degenerated cells into the sinusoid was observed. On the 124th day, multiple formation of nodule was found in the lobules. The nodules were observed increasing in size gradually with the progress of the disease on the 207th, 294th, 356th and 392nd days. Among the cell components, lymphoid cells and histiocytic cells were conspicuous. Histiocyte reaction became remarkable as death drew near. Deposition of hemosiderin granules was also very striking.

Post-mortem histopathological examination revealed activation of cells of the reticulo-endothelial system of the whole body, lymphoid-cell reaction in the spleen and the lymph nodes, and degeneration of parenchyma.

Case 2, BOTAN by name, female, adult

This mare was injected subcutaneously with 50 ml of emulsion of aborted fetus derived from SHIKISHIMA (Case 5 in Sect. C-2 above), a naturally infected case. The first pyrexia was observed on the 44th day of the disease. After that, recurrent fever was shown three times. This case was subjected to vital staining (12 doses totaling 2,050 ml daily) and sacrificed on the 113th day of the disease.

Findings in spleen punctate The λ-cell, monocyte, and reticulo-endothelial-cell reactions were observed from the 30th to the 92nd day of the disease. The λ-cell reaction became inconspicuous on and after the 97th day of the disease.

Findings in peripheral blood Neutrophilia was shown temporarily after the inoculation of the emulsion. Erythrocyte and leucocyte counts did not fluctuate remarkably at the time of the first pyrexia, but the erythrocyte sedimentation rate was increased. Siderocyte test turned positive on the 44th day of the disease.

Findings in liver tissue No particular changes were observed on the 44th and 97th days of the disease. On the 100th day, endothelial cells swelled and increased in number, and sloughing of a small number of degenerated cells was found in the sinusoids. On the 104th day of the disease, formation of nodules of small size was to be seen in the lobules.

Post-mortem histopathological examination revealed lymphoid-cell reaction in the spleen and the lymph nodes. Pathological changes of the liver were the same at autopsy as at biopsy.

Summary

It has been made clear that the investigation of λ-cell reaction in spleen punctate constitutes a part of essential studies on the pathological changes caused by EIA. In order to know the pathological role of λ-cell reaction, it is one of the effective methods to observe the whole course of the disease from the onset to death.

In the observation of two experimental cases, it was necessary to divide the

course of the disease into the beginning, chronic, and relapsing stages from the characteristics of the pathological changes. The first case succumbed on the 397th day of the disease, passing through all these stages. The second case was sacrificed at the beginning of the chronic stage (on the 113th day).

Beginning stage This stage was started with the typical pyrexia after the incubation period. In the spleen punctate, λ-cell, monocyte, and reticulo-endothelial-cell reactions were outstanding. These reactions were noted on the 30th day of the disease prior to the appearance of the first pyrexia in the second case. They were observed on the 41st day in the first case, but since no puncture was performed earlier than that date, it was obscure whether this was the first appearance of these reactions or not. In the liver tissue obtained by puncture, no particular changes were found at the time of the first pyrexia, but localized swelling and sloughing of endothelial cells were observed on the 63rd (in the first case) and 100th day (in the second case) of the disease. In the peripheral blood, erythrocyte count decreased at the time of the first pyrexia, but recovered to normal soon and then decreased again gradually. Leucocyte count decreased temporarily and recovered to normal soon, but showed increase after that. Siderocyte tests turned positive for the first time on the 41st and 44th days of the disease in the first and second cases, respectively.

Chronic stage In the spleen punctate, λ-cell and reticulo-endothelial-cell reactions were not observed any more, but monocyte reaction was present constantly. Nucleated-cell count was increasing. The characteristic of the liver tissue lesion was multiple formation of nodule having developed gradually. The reacting cells filling the nodules were mostly lymphoid cells. Such change was observed on the 124th and 104th days of the disease in the first and second cases, respectively. In the peripheral blood, erythrocytes were gradually decreasing to show anemia while leucocytes were increasing. The differential count of leucocytes revealed neutrophilia in the febrile period and lymphocytosis in the non-febrile period. The amount of siderocytes detected was larger than at any other time in the whole course of the disease. In the bone-marrow punctate, mature cells of the granulocyte group showed some increase. Monocyte reaction was also present.

Relapsing stage The signs of anemia were prominent in the spleen punctate. Although the activation of cells of the reticulo-endothelial system was conspicuous in the post-mortem histopathological sections, on such quantitative abnormalities as would predict the post-mortem pathological changes were observed in the ante-mortem puncture specimens, which contained only scattered macrophages. In the liver tissue, nodules were growing gradually; histiocytic cells and

macrophages participated in them conspicuously. In the peripheral blood, anemia was indicated in high degree, leucocytes were increasing, and the erythrocyte sedimentation rate was always high. The amount of siderocytes detected was somewhat smaller in this stage than in the chronic stage. The bone-marrow punctate revealed a decrease in number of cells of erythroblast group. Besides, clinically, emaciation was remarkable, the peripheral part of the body became edematous, and high fever was continued. Post-mortem histopathological changes included activation of cells of the reticulo-endothelial system in the whole body, lymphoid-cell reaction in the spleen and the lymph nodes, and degeneration of parenchyma.

2 Cases of short-term observation (Tables 5~10, Plate IV)

Report of cases

Case 1, No. 18, male, 2 years of age

This horse was inoculated subcutaneously with 10 ml of sodium-citrated blood from a naturally infected case. It manifested pyrexia on the 9th day and was sacrificed on the 13th day. No characteristic pathological changes were observed at any site in either ante- or post-mortem examinations.

Case 2, No. 24, female, 2 years of age

This mare was inoculated subcutaneously with 5 ml of sodium-citrated bone-marrow punctate of horse No. 19, a naturally infected case and 10 ml of sodium-citrated blood of horse No. 21, an experimentally infected case. Pyrexia was shown on and after the 8th day of the disease. The spleen punctate revealed λ-cell, monocyte, and reticulo-endothelial-cell reactions on and after the 8th day of the disease. No pathological changes of the liver were observed before death. Reactions in the peripheral blood were somewhat conspicuous. This mare was sacrificed on the 14th day. Post-mortem histopathological examination revealed activation of cells of the reticulo-endothelial system in the whole body and λ-cell reaction in the spleen and the lymph nodes.

Case 3, No. 25, female, adult

This mare was inoculated subcutaneously like the preceding one with 5 ml of sodium-citrated bone-marrow punctate of horse No. 19, a naturally infected case, and 10 ml of sodium-citrated blood of horse No. 21, an experimentally infected case. Pyrexia was shown on and after the 11th day of the disease. The spleen punctate revealed λ-cell reaction on and after the 10th day and λ-cell, monocyte, and reticulo-endothelial-cell reactions on and after the 17th day of the disease. Pathological changes were noted in the liver on the 21st day (multiple formation of necrobiotic foci). Reactions in the peripheral blood were a little

obscure. This mare was sacrificed on the 22nd day. Post-mortem histopathological findings included activation of cells of the reticulo-endothelial system in the whole body, λ-cell reaction in the spleen and the lymph nodes, and degeneration of parenchyma.

Case 4, No. 23, female, adult

This mare was inoculated subcutaneously like the preceding two with 5 ml of sodium-citrated bone-marrow punctate of horse No. 19, a naturally infected case, and 10 ml of sodium-citrated blood of horse No. 21, experimentally infected case.

TABLE 5. *Splenogram of Horse No. 24*

	BEFORE INOCULATION	DAYS AFTER INOCULATION				
		3	8	10	13	14
Erythrocytes (10^6/mm^3)	9.40	15.15	12.75	12.20	9.35	9.35
Nucleated cells (10^3/mm^3)	25.4	68.0	39.0	25.6	22.4	49.0
Lymphatic cells	%	%	%	%	%	%
Lymphoblasts	0	0.6	0.2	0.4	0.4	0.4
Large lymphocytes	10.2	16.8	6.0	16.4	11.2	14.8
Small lymphocytes	38.6	50.8	42.0	25.4	35.8	35.2
Total	48.8	68.2	48.2	42.2	47.4	50.4
Basophil round cells	0.6	1.6	4.0	4.2	4.6	13.8
Monocytes						
Juvenile form	3.0	8.0	12.0	12.6	10.4	13.8
Mature form	2.8	3.6	9.0	5.4	6.2	6.2
Total	5.8	11.6	21.0	18.0	16.6	20.0
Reticulo-endothelial system						
Reticulum cells	0.4	1.2	0.6	5.8	3.4	2.0
Round-transformed cells	0.2	4.0	6.2	6.0	6.2	2.6
Siderocytes	0.2	1.2	1.2	1.2	0.8	0.8
Endothelial cells	0	0	0.4	0	0	0
Total	0.8	6.4	8.4	13.0	10.4	5.4
Neutrophil leucocytes	42.8	12.0	17.0	21.8	19.8	10.2
Eosinophil leucocytes	0.6	0.2	0	0	0.6	0
Basophil leucocytes	0	0	0.2	0.4	0	0
Unidentified cells	0	0	1.0	0	0	0
Karyorrhexis	0.6	0	0.2	0.2	0.6	0.2
Mitosis	0	0	0	0.2	0	0

Pyrexia was shown on and after the 12th day of the disease. In the spleen punctate, λ-cell, monocyte, and reticulo-endothelial-cell reactions were observed on and after the 17th day of the disease. Pathological changes were observed in the liver also on the 17th day (spherical transformation of stellate cells of KUPFFER and wandering of histiocytic cells in the sinusoids). Reactions in the peripheral blood were slightly conspicuous. This mare was sacrificed on the 22nd day. Post-mortem histopathological examination revealed activation of cells of the reticulo-endothelial system in the whole body and λ-cell reaction in the spleen and the

TABLE 6. *Splenogram of Horse No. 25*

	BEFORE INOCULATION	DAYS AFTER INOCULATION	
		10	20
Erythrocytes (10^6/mm^3)	10.25		
Nucleated cells (10^3/mm^3)	54.6		
Lymphatic cells	%	%	%
Lymphoblasts	0	0.2	0.2
Large lymphocytes	21.6	14.4	6.4
Small lymphocytes	57.2	62.4	30.4
Total	78.8	77.0	37.0
Basophil round cells	1.0	5.2	7.4
Monocytes			
Juvenile form	3.2	3.4	7.8
Mature form	2.4	3.2	7.4
Total	5.6	6.6	15.2
Reticulo-endothelial system			
Reticulum cells	1.2	1.8	4.0
Round-transformed cells	1.2	1.2	3.4
Siderocytes	3.0	1.4	0.4
Endothelial cells	0	0	0
Total	5.4	4.4	7.8
Neutrophil leucocytes	7.2	5.8	31.2
Eosinophil leucocytes	1.8	0.6	0.8
Basophil leucocytes	0.2	0.4	0.2
Unidentified cells	0	0	0
Karyorrhexis	0	0	0.2
Mitosis	0	0	0.2

lymph nodes.

Case 5, No. 17, male, 2 years of age

This horse was inoculated subcutaneously with 10 ml of sodium-citrated blood of a naturally infected horse. A slight pyrexia was shown on and after the 20th day of the disease. Examination of spleen punctate revealed λ-cell and monocyte reactions on and after the 5th day. No pathological changes of the liver were observed before death. Reactions in the peripheral blood were somewhat obvious. This horse was sacrificed on the 23rd day. Post-mortem histopathological

TABLE 7. *Splenogram of Horse No. 23*

	BEFORE INOCULATION	DAYS AFTER INOCULATION			
		13	17	20	21
Erythrocytes (10^6/mm^3)	11.80	14.90			
Nucleated cells (10^3/mm^3)	24.0	85.0			
Lymphatic cells	%	%	%	%	%
Lymphoblasts	0.2	0	0	0	0
Large lymphocytes	26.2	4.0	11.6	8.2	6.0
Small lymphocytes	30.2	41.6	25.6	31.4	37.0
Total	56.6	45.6	37.2	39.6	43.0
Basophil round cells	1.8	2.4	19.8	19.8	22.8
Monocytes					
Juvenile form	3.2	10.2	16.6	16.8	5.6
Mature form	2.6	5.6	5.2	4.6	2.2
Total	5.8	15.8	21.8	21.4	7.8
Reticulo-endothelial system					
Reticulum cells	0.8	1.6	6.0	3.4	9.6
Round-transformed cells	1.4	4.8	6.8	4.8	10.4
Siderocytes	3.6	1.6	0.2	0	0
Endothelial cells	0	0	0	0	0
Total	5.8	8.0	13.0	8.2	20.0
Neutrophil leucocytes	24.0	27.0	6.2	10.8	6.2
Eosinophil leucocytes	2.2	0	0	0	0
Basophil leucocytes	0.4	0.2	0	0	0
Unidentified cells	0	0	0	0	0
Karyorrhexis	3.4	0.8	1.8	0	0
Mitosis	0	0.2	0.2	0.2	0.2

examination disclosed activation of cells of the reticulo-endothelial system in the whole body, λ-cell reaction in the spleen and the lymph nodes, and degeneration of parenchyma.

Case 6, No. 22, female, adult

This mare was inoculated subcutaneously with 50 ml of sodium-citrated blood of horse No. 19, a naturally infected case. A slight pyrexia was shown on and after the 17th day of the disease. In the spleen punctate, λ-cell, monocyte and reticulo-endothelial-cell reactions were observed on and after the 22nd day, but

TABLE 8. *Splenogram of Horse No. 17*

	BEFORE INOCULATION	DAYS AFTER INOCULATION			
		5	13	20	23
Erythrocytes (10^6/mm^3)	3.85	5.45	6.37	5.27	
Nucleated cells (10^3/mm^3)	24.0	42.6	42.8	38.4	
Lymphatic cells	%	%	%	%	%
Lymphoblasts	3.2	0.2	1.0	2.2	0
Large lymphocytes	8.0	7.4	11.8	5.6	8.6
Small lymphocytes	56.6	29.4	42.4	45.4	51.4
Total	67.8	37.0	55.2	53.2	60.0
Basophil round cells	2.4	11.8	3.2	4.0	16.8
Monocytes					
Juvenile form	7.6	16.4	17.4	13.6	10.4
Mature form	3.0	7.6	11.6	12.4	2.6
Total	10.6	24.0	29.0	26.0	13.0
Reticulo-endothelial system					
Reticulum cells	1.6	0	0.6	1.8	0.6
Round-transformed cells	1.2	3.0	1.6	3.2	0.8
Siderocytes	0.6	0.6	0.2	0.2	0
Endothelial cells	0	0	0	0	0
Total	3.4	3.6	2.4	5.2	1.4
Neutrophil leucocytes	14.0	21.6	8.8	10.6	7.6
Eosinophil leucocytes	1.0	1.0	0.2	0.2	0.6
Basophil leucocytes	0.2	0	0.2	0	0
Unidentified cells	0	0.6	0.4	0.8	0
Karyorrhexis	0.2	0.2	0.6	0	0.2
Mitosis	0.4	0.2	0	0	0.4

they were sometimes intermittently observed. Pathological changes were formed in the liver on the 29th day (small-sized nodule formation). There are some slight reactions in the peripheral blood. This mare was sacrificed on the 32nd day. Post-mortem histopathological examination showed λ-cell reaction in the spleen and formation of small-sized nodules in the liver.

Case 7, No. 21, female, adult

This mare was inoculated subcutaneously with 50 ml of sodium-citrated blood of horse No. 19, a naturally infected case. A slight fever was shown on and

TABLE 9. *Splenogram of Horse No. 22*

	BEFORE INOCULATION	DAYS AFTER INOCULATION						
		5	17	22	24	25	29	32
Erythrocytes (10^6/mm^3)	7.37	18.00	12.15	11.21	16.30	14.40	14.55	14.80
Nucleated cells (10^3/mm^3)	24.0	80.0	29.8	24.2	31.0	41.0	89.0	54.0
Lymphatic cells	%	%	%	%	%	%	%	%
Lymphoblasts	1.0	0	0.4	0.6	0.2	1.2	0.8	0.8
Large lymphocytes	14.8	2.4	10.6	8.8	12.0	9.6	11.6	13.4
Small lymphocytes	58.6	54.8	36.8	33.2	46.8	43.4	42.0	39.2
Total	74.4	57.2	47.8	42.6	59.0	54.2	54.4	53.4
Basophil round cells	1.4	0.8	1.2	6.6	2.6	4.2	3.6	1.6
Monocytes								
Juvenile form	4.4	6.0	14.6	14.2	12.8	17.8	16.0	17.4
Mature form	5.8	6.4	10.0	8.4	7.4	7.4	6.2	6.2
Total	10.2	12.4	24.6	22.6	20.2	25.2	22.2	23.6
Reticulo-endothelial system								
Reticulum cells	2.6	0	0.2	3.0	1.2	1.4	1.8	2.2
Round-transformed cells	1.4	6.8	5.4	5.6	5.2	3.6	5.6	4.6
Siderocytes	0.6	2.8	2.0	1.6	1.0	0.6	2.8	2.4
Endothelial cells	0	0	0	0	0.2	0	0	0
Total	4.6	9.6	7.6	10.2	7.6	5.6	10.2	9.2
Neutrophil leucocytes	7.2	18.0	16.2	13.0	9.2	8.0	7.2	9.6
Eosinophil leucocytes	0.8	0.8	0.4	0.8	0.2	0.6	1.2	0.6
Basophil leucocytes	0.2	0	0.2	0	0.2	0.4	0.2	0.4
Unidentified cells	0	1.2	1.4	3.6	1.0	1.2	0.8	1.2
Karyorrhexis	1.2	0	0.6	0.6	0	0.4	0	1.4
Mitosis	0	0	0	0	0	0.2	0.2	0

TABLE 10. *Splenogram of Horse No. 21*

	BEFORE INOCULATION	DAYS AFTER INOCULATION		
		17	33	39
Erythrocytes (10^6/mm^3)	14.40	15.00	14.20	12.25
Nucleated cells (10^3/mm^3)	39.6	30.0	49.0	82.0
Lymphatic cells	%	%	%	%
Lymphoblasts	0	0	0.6	0
Large lymphocytes	0.8	11.0	11.6	10.6
Small lymphocytes	50.2	33.2	21.0	23.6
Total	51.0	44.2	33.2	34.2
Basophil round cells	0.8	0.8	11.6	6.0
Monocytes				
Juvenile form	3.0	12.0	16.4	28.4
Mature form	4.2	9.0	9.2	10.0
Total	7.2	21.0	25.6	38.4
Reticulo-endothelial system				
Reticulum cells	1.0	0.4	0.4	0.2
Round-transformed cells	0.6	2.0	2.0	2.8
Siderocytes	3.6	1.0	0.4	1.0
Endothelial cells	0	0	0	0
Total	5.2	3.4	2.8	4.0
Neutrophil leucocytes	33.2	28.4	24.6	16.6
Eosinophil leucocytes	2.0	1.6	0.6	0
Basophil leucocytes	0.4	0.4	0.4	0.2
Unidentified cells	0	0	0.8	0.4
Karyorrhexis	0.2	0.2	0.4	0.2
Mitosis	0	0	0	0

after the 30th day of the disease. In the spleen punctate, λ-cell and monocyte reactions appeared on and after the 33rd day. No pathological changes of the liver were observed before death. Reactions in the peripheral blood were unclear. This mare was sacrificed on the 41st day. Post-mortem histopathological changes included formation of small-sized nodules in the liver and λ-cell reaction in the spleen.

Summary

Following the long-term observation described in the preceding section D–1,

observations were made on 7 cases with regard to pathological changes manifested at the early stage of infection (pathological changes of the beginning stage).

Of these changes, those in the spleen punctate were the most characteristic and common to all the cases. The λ-cell, monocyte, and reticulo-endothelial-cell reactions were noticeable in the spleen punctate. The λ-cell reaction was the most characteristic of them. The date of the disease when the λ-cell reaction was observed for the first time was the 5th day in case 5, the 8th day in case 2, the 10th day in case 3, the 17th day in case 4, the 22nd day in case 6, the 30th day in case 2 of section D–1, and the 33rd day in case 7. Of these cases listed, cases 3, 5 of section D–2, and 2 of section D–1 showed this reaction during the period of incubation. The duration of the reaction was 6 to 19 days even in the cases under short-term observation and 84 days (case 1 of section D–1) and 62 days (case 2 of section D–1) in the cases under long-term observation.

As for mother tissue, λ cells multiplied around the arterioles in the spleen. Reticulum cells multiplied diffusely or locally in the splenic pulp. Both λ cells and reticulum cells constituted the principal pathological changes in the histological view.

The pathological changes shown in the liver were divided into several forms. They were the form of diffuse spherical transformation of stellate cells of KUPFFER (case 2 on the 14th day of the disease and case 4 on the 17th day), that of multiple formation of necrobiotic foci (case 3 on the 21st day and case 5 on the 23rd day), that of small-sized nodule formation (case 6 on the 29th day and case 7 on the 41st day), and that of nodule formation (case 1 of section D–1 on the 124th day and case 2 of section D–1 on the 104th day).

When the spleen and the liver pathological changes were compared, λ-cell reaction appeared earlier in the spleen of one case in which formation of degenerative foci and activation of cells of the reticulo-endothelial system were observed in the liver than in the spleen of any other case. It is also clear that the pathological changes appeared earlier in the spleen than in the liver.

As for reactions shown in the peripheral blood, the decrease in erythrocyte and leucocyte counts and the rise in the erythrocyte sedimentation rate at the time of the first pyrexia were conspicuous. They were particularly outstanding in those cases which were under severe attack of disease. Siderocyte detection showed a tendency to be small in amount at the early stage of infection. No characteristic changes were observed in the bone-marrow punctate.

As for the type of fever, continuous fever was shown in case 2. In other cases, there was a tendency of fever to come to a crisis in several days. The febrile condition, however, was not clear in some other cases.

Chapter IV

DISCUSSION

Reliability of technics

It has already been mentioned by other investigators that the changes in the spleen have an important significance in the post-mortem diagnosis of EIA. Scrupulous consideration should be given to the problem of what changes of the spleen are detected by what method at the time of ante-mortem examination.

It is impossible at present to harvest a necessary amount of tissue from the spleen, differently to the case of the liver. Accordingly, there is no other method than to analyze the cell components of a specimen collected by puncture. The parallel relationship between the findings in tissue and those in punctate of the spleen has already been described above, but further fundamental discussion is required on this point.

The spleen has important functions of blood formation, iron treatment, and protection against foreign bodies. Morphologically, it is an organ combining portions which correspond to the respective functions. Therefore, the diagnosis of the spleen by puncture should be made on the basis of earlier study on the relationship between the structure and components of the organ and the fluid obtained by puncture. Histology indicates that the spleen is composed of the capsule, trabeculae, follicles, and red pulp. Of these, the capsule, trabeculae, and system of blood vessels form a strong structural tissue or are in very close relations to it and, since not participants to absorption, may be excluded from the examination of specimens obtained by puncture.

The direct objects of study by puncture are the components of the follicle (F), the components of the splenic pulp (P), exclusive of follicles, and the contents of the blood vessels (S) (chiefly the contents of the splenic sinus); in the sample actually obtained by puncture the material is a mixture of the three.

In the materials from puncture, two groups for comparison are considered to be present. They are F and P (above) containing a large amount of the proper components of the spleen and S which is strongly influenced by the peripheral blood components. The presence of these groups can be demonstrated from experience and statistical study and must first be taken into consideration as an important condition in the formation of an over-all spleen puncture.

The author calls the former "splenic pulp blood" and the latter "splenic sinus

blood".

When the spleen punctate is studied, from this point of view, in specimens collected by performing spleen puncture several times, the actual composition of cells of the spleen can be clearly ascertained in a certain individual. In consequence, although spleen puncture is a method of investigating the spleen indirectly by sucking up a portion of the organ as a fluid specimen, this method makes it possible to presume the actual composition of cells of the spleen before death because of the homology of the specimen obtained by puncture with the organ itself.

Characteristics of findings in the spleen punctate and the relationship between the stage of disease and pathological changes in equine infectious anemia (Table 11 & Text figure 2)

The relationship between the stage of disease and pathological changes must always be taken into consideration when the spleen punctate is studied in EIA.

The numerical values of the various studied items in spleen punctate are clearly different a priori and statistically not only between normal and infected animals but also between such stages of disease as the beginning, chronic, and relapsing stages.

Beginning stage The number of erythrocytes is large, but that of nucleated

TABLE 11. *Comparison of Splenograms in the Beginning and the Chronic Stage of Equine Infectious Anemia*

	NORMAL CONTROL		EQUINE INFECTIOUS ANEMIA			
			Beginning		Chronic	
	Pulp	Sinus	Pulp	Sinus	Pulp	Sinus
Erythrocytes (10^6/mm^3)	10.84	8.73	12.22	9.44	9.57	7.87
Nucleated cells (10^3/mm^3)	32.7	16.0	42.6	17.0	52.0	20.4
	%	%	%	%	%	%
Lymphatic cells	70.68	39.95	52.94	28.66	57.42	29.17
Basophil round cells	0.92	0.30	4.63	1.05	1.71	0.41
Monocytes	4.15	4.63	17.61	12.20	15.25	13.80
Cells of Reticulo-endothelial system	3.68	1.60	6.65	3.98	4.31	2.25
Neutrophil leucocytes	18.63	50.23	17.08	51.30	19.29	52.06
Eosinophil leucocytes	1.11	2.30	0.31	0.95	0.59	0.79
Basophil leucocytes	0.15	0.53	0.10	0.24	0.14	0.10
Unidentified cells	0.68	0.46	0.66	1.61	1.28	1.42
Mitosis	0	0	0.02	0.01	0.01	0

EXPLANATION OF TEXT FIGURE 2

Diagrams of the pathological changes of the spleen

A: normal spleen

B: spleen at the beginning stage of disease

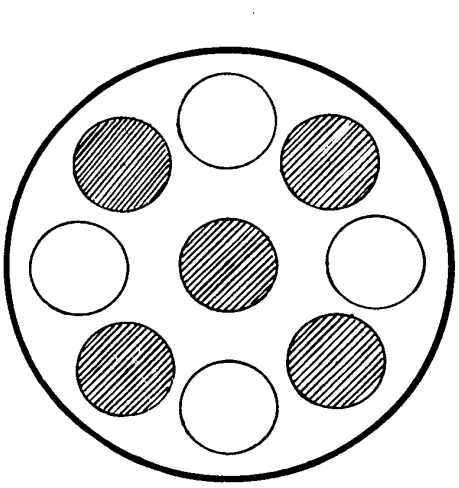
C: spleen at the chronic stage of disease

D: spleen at the relapsing stage of disease

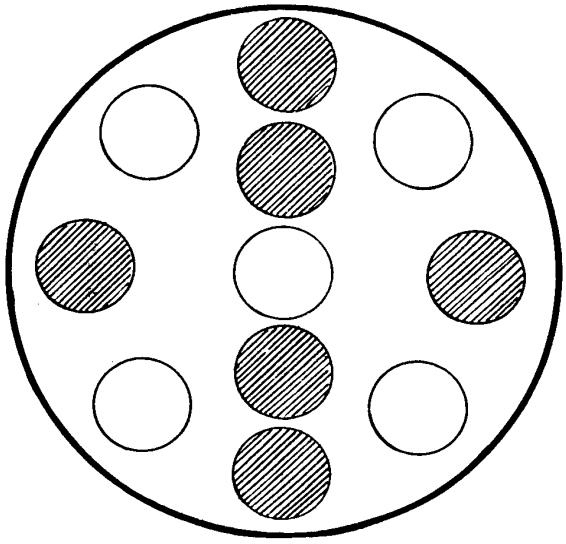
◍: erythrocyte, one solid circle representing 2 million erythrocytes

○: nucleated cell, one hollow circle representing 8 thousand nucleated cells

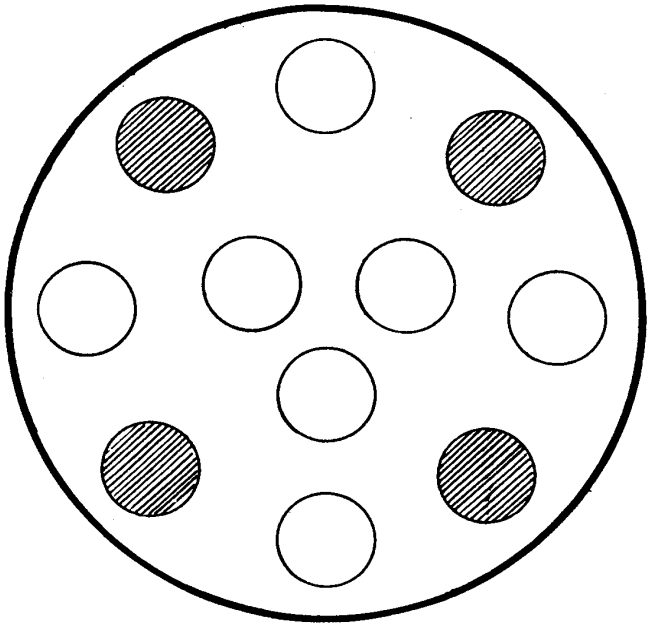
TEXT FIG. 2.



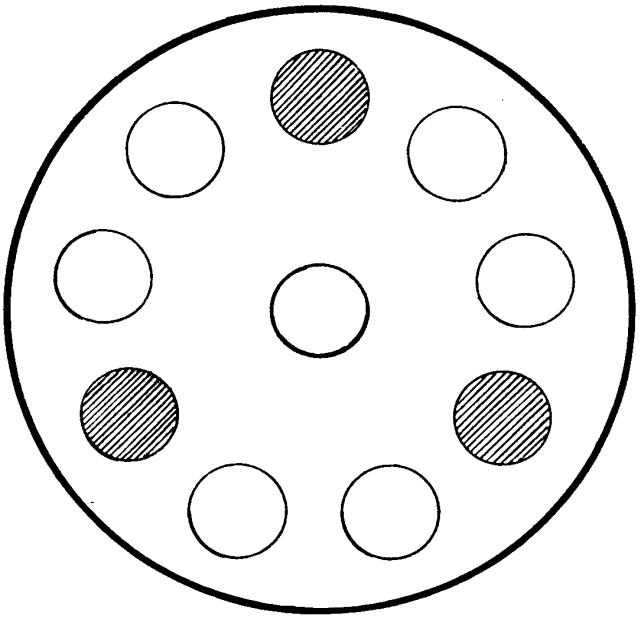
A



B



C



D

cells is not very different from the value in the normal period. Accordingly, it is chiefly due to the changes in the circulating blood that the spleen increases macroscopically in size at this stage. Such changes seem to consist of the detention of erythrocytes, stagnation of the amorphous components, and other local disturbances of circulation. Lambda cells, monocytes, and reticulo-endothelial cells are all increasing.

OHSHIMA (1954) studied the lymph nodes of many naturally infected cases and concluded that the "L" cells showed the most active reaction in the subacute type of the disease. This fact gives some suggestion with regard to the course of the subacute type after infection.

Chronic stage It is infrequent that erythrocytes decrease noticeably in number. Nucleated cells, however, increase in number (hyperplasia of nucleated cells). Monocytes are large in number in the differential count. The number of λ cells is large in some cases, but it is smaller than at the beginning stage. A high λ-cell count is not common to all the cases. Therefore, although the absolute value of the nucleated cell count is high, no ratio is particularly high, except that of monocytes. This is quite possibly due to the very high absolute value of the number of those cells which are designated as "the cells of lymphocyte group" by the author (which include those described as lymphoid cells in histopathological specimens) and which supply a majority of the cells in the differential count. As mentioned above, this phenomenon has already been observed by SAKAI and TAMURA (1953). Different from the beginning stage, it is mostly due to hyperplasia of nucleated cells at the chronic stage that the organ increases macroscopically in size.

Relapsing stage Erythrocytes decrease in number (systemic anemia). Lambda cells do not react again. Other changes are similar to those at the chronic stage.

Diagnostic value of comprehensive information on the spleen punctate

It has been demonstrated that EIA is an infectious disease caused by a virus, but no specific microbiological reaction has been established. Siderocyte detection and liver puncture are at present the diagnostic methods which are considered effective.

The siderocyte detection is based on the phagocytic function and is most widely used in Japan nowadays. This method has the advantage in its simple technic, accurate results, and applicability to the entire course of the disease, but it has a disadvantage in respect to the frequent fluctuation of the appearance of siderocytes in the peripheral blood.

The liver puncture method is based on the characteristic histopathological

changes of the liver. The character of the pathological changes is such that the value of this method is almost perfect in diagnosis if liver tissue can be harvested in and after the chronic stage (atrophy of the right lobe is infrequently shown), but it is not seldom that this method becomes worthless for a certain period of time during the beginning stage of infection, because the liver tissue shows usually no characteristic changes for such a period.

The spleen puncture method makes presumption of the pathological changes of the spleen on the basis of the composition of cells in the spleen punctate harvested. It might be unnecessary to discuss the characteristics of the findings in the spleen punctate and the reliability of the technic here again. When this method is applied in diagnosis, some reasonable conditions must be taken into consideration from a general point of view. For example, careful preparation is required prior to a study on the relationship between reacting cells and their mother tissue. Next, it is of little value to compare only relative increase and decrease of reacting cells. It is desirable to use as criteria those cells which have relationship with the histopathological changes of the spleen, have a clear and well-defined cytological appearance, and have been fully analyzed pathologically.

Among the characteristic cells in the over-all view of spleen punctate in EIA, λ cells are those which best satisfy these conditions.

The spleen puncture method, which uses the λ cell as criterion for diagnosis, is effective only at the beginning stage. In other words, it is applicable only as a method for early diagnosis. For this reason, it excels the other two methods.

Theoretically, the spleen puncture method is effective for diagnosis even in and after the chronic stage or regardless of the stage of disease. However, as cytological studies on lymphoid cells are still insufficient, it is difficult to recognize any objective value of this method when it is employed for diagnosis. For the time being, it is rather simple and speedy to make diagnosis by means of the liver puncture method.

As stated above, the spleen and liver puncture methods have been established on the basis of histopathological changes and mutually supplement the respective disadvantageous points of each other. Therefore, when these methods are employed their advantageous points should be utilized by taking the features of each of the two methods into consideration.

In differential diagnosis, the reaction of such cells as λ cells is scarcely observed in histopathological changes of other infectious disease in horses. Equine paratyphoid prevailing in the Hokkaido and Tohoku regions is in foals considered as systemic typhoid. In such diseases, however, no reaction of such cells has been proved in the spleen by KUTII et al. (1947) and by HAMADA (1951).

In further consideration, those cases which are possibly the most difficult to distinguish from EIA may be leucemia and neoplasms in the spleen originated from plasma cells or juvenile lymphocytes. Such cases in references and experiences, however, are very seldom met with. Even if they are encountered, it may be possible to discriminate them from EIA from the general conditions.

Chapter V

SUMMARY

1. The author made attempts to grasp the characteristics of the pathological changes of the spleen in cases of equine infectious anemia (EIA) by means of the puncture method.

2. The normal control data on spleen punctate were obtained from puncture on 20 healthy horses (49 specimens in total). In addition, control data on spleen punctate and bone-marrow punctate were obtained from 15 horses kept for the production of equine paratyphoid immune serum (24 specimens in total).

The EIA group consisted of 16 naturally and 9 experimentally infected horses. Ante-mortem observations on spleen punctate, liver tissue by puncture, bone-marrow punctate, and peripheral blood were made on them continually in the course of the disease. Upon death they were subjected to autopsy.

3. The naturally infected cases showed two types of spleen punctate, those with and those without λ-cell reaction. When studied histopathologically, the spleen with λ-cell reaction in its punctate specimen was noted to present this reaction in its tissue section. Continual observations performed in the course of the disease made it clear that λ-cell reaction was remarkable in the specimens of spleen punctate and tissue of the cases of subacute type, but that it did not appear in those of the cases of chronic and relapsing types.

4. The following observations were made on the experimentally infected cases.

Long-term observation Pathological changes were studied continually through the entire course from onset of disease to death in two cases inoculated with viral material. One case died on the 397th day of the disease and the other was sacrificed on the 113th day.

The outstanding characteristic changes were λ-cell reaction in the spleen punctate at the beginning stage, formation of nodule in the liver at the chronic stage, and degeneration of parenchyma and activation of cells of the reticulo-endothelial system almost in whole body at the relapsing stage.

From these observations, attention is called to the presence of the stage in close relation to the pathological changes and to the condition of the spleen punctate shown at the beginning stage of the disease.

Short-term observation Observation was made on pathological changes appearing in the early period (changes at the beginning stage) after inoculation

of viral material.

Of the changes at the beginning stage, those which were found in the spleen punctate were especially characteristic and common to all the cases studied. They are λ-cell, monocyte, and reticulo-endothelial-cell reactions.

The pathological changes of the liver included activation of cells of the reticulo-endothelial system, degeneration of parenchyma, and formation of nodules. The occurrence of the significant pathological changes came later in the liver than in the spleen.

Siderocytes in the peripheral blood were small in amount and frequently were difficult to detect. No particularly remarkable changes were to be seen in the bone-marrow punctate.

5. The following characteristics were observed in the spleen punctate at different stages of EIA.

Beginning stage Erythrocytes were large in number (hyperemia of splenic pulp). Lambda-cell, monocyte, and reticulo-endothelial-cell reactions were present.

Chronic stage Nucleated cells were large in number (hyperplasia of nucleated cells).

Relapsing stage Erythrocytes were small in number (anemia) and nucleated cells large in number (hyperplasia of nucleated cells).

6. Characteristics of λ cells The cytoplasm was wide, well-defined, and uniformly strongly basophilic. It sometimes had a localized unstained portion and very seldom contained a vacuole. The nucleus was centrally located and spherical. When fixed and stained, it presented no depression, indentation nor lobulation. Nuclear division, mitotic and amitotic, was seen frequently. There were many cells of large size. Some large cells had nucleoli. Phagocytic function was seldom met with.

The mother tissue of λ cells was found in such organs with lymphoid tissue as the spleen or the lymph nodes.

In the spleen, the mother tissue was in the perivascular tissue of the arterioles of splenic pulp.

Lambda cells are present in the normal spleen in so small a quantity that they are negligible in number in the spleen punctate. They appeared in largest number at the beginning stage of EIA infection. As the disease became chronic, they decreased in number gradually, being undetectable at last. Even when the course of the disease became relapsing and severe symptoms were manifested, there was no tendency of recurrence of λ-cell reaction.

7. When spleen puncture is used as a means of diagnosis, λ cells are qualified to be employed as a criterion of diagnosis. In addition, spleen puncture is effective as a method of early diagnosis.

APPENDIX

Procedure of Spleen Puncture as Employed for Diagnosis

The animal to be punctured is not allowed to have severe exercise before and after operation. It can be used for light work the day following puncture. The horse is held in the right standing position and the point of puncture is chosen on a line passing the hip joint at the anterior border of the last rib on the left side. The assistant shaves this portion previously to the extent of about 3 cm^2, washes it with positive soap or saponated cresol solution, and paints it well with tincture of iodine. (It is recommended that puncture needle be placed in a test tube tightly sealed and be sterilized with dry heat or in the autoclave beforehand.) After washing his fingers thoroughly, the operator sticks the puncture needle into the skin at a right angle and makes it reach the spleen with elastic response. After pushing the needle into the spleen, the operator removes the inner needle and sucks up the spleen punctate in the outer sheath, by means of a 10-ml syringe with a dried cylinder. The optimum amount of spleen punctate is 0.1 to 0.2 ml. (In no case, should the amount of a sample be larger than necessary in order to protect the health of the animal and to obtain an accurate cell count.) The outer sheath of the puncture needle is removed with the syringe cylinder attached. The sample in the sheath is blown out on a clean slide to prepare smears. Every step must be carried out rapidly as spleen punctate coagulates quickly. After the operation, it is enough to paint the site of the puncture with tincture of iodine. Smears are dried in the air and stained. At first a mixture of equal amounts of May-Grünwald solution and methanol is placed on the smear. After 5 minutes it is washed off with water and the smear is stained with Giemsa solution for 30 minutes. Then the smear is washed with water, dried, and microscoped with oil immersion apparatus. After it is ascertained that the smear is that of splenic pulp blood, differential count is performed on 500 to 1,000 nucleated cells and a positive diagnosis is made when "basophil round cells" exist at a rate of more than 3 per cent.

Characteristics of splenic pulp blood When sucked up, it comes into the cylinder slowly. It is viscous and quick to coagulate. The number of nucleated cells is large. Lymphocytes are larger in number than neutrophil leucocytes in the differential count. If the sample is found to have been harvested from splenic sinus blood, puncture must be performed again as it is unfit for diagnosis.

REFERENCES

1) DOBBERSTEIN, J. (1934): *Berl. tierärztl. Wschr.*, **50**, 192.
2) HAMADA, S. (1951): *Mem. Fac. Agric. Hokkaido Uuiv.*, **1**, 45 (in Japanese with English summary).
3) ICHIKAWA, O., R. ISHITANI, D. SHIBATA, Y. OGURA, M. TAKANAMI, T. HORIUCHI & T. YONEMURA (1955): Exp. Rep. No. 29 Gov. Exp. Sta. Anim. Hyg., 131.
4) ISHII, S. & N. NAKAMURA (1942): Exp. Rep. No. 19 Gov. Exp. Sta. Anim. Hyg., 112 (in Japanese).
5) ISHII, S., K. NOBUTO & K. TANAKA (1940): *Jap. J. vet. Sci.*, **2**, 531 (in Japanese with English summary).
6) ISHII, S. & E. TABUCHI (1942): Exp. Rep. No. 19 Gov. Exp. Sta. Anim. Hyg., 250 (in Japanese).
7) KUTII, T., H. SUTO & T. SHOHO (1946): *Jap. J. vet. Sci.*, **8**, 107 (in Japanese with English summary).
8) MIURA, S. (1952): *Ibid.*, **14**, 489 (in Japanese).
9) MIURA, S. & I. WADA (1943): *Jap. J. mil. vet. Ass.*, No. 413, 1215 (in Japanese).
10) OHSHIMA, K. (1954): *Jap. J. vet. Res.*, **2**, 73.
11) SAKAI, T. & S. TAMURA (1953): *J. Jap. vet. med. Ass.*, **6**, 84 (in Japanese).
12) SUGANO, S., M. TAJIMA & M. OHBAYASHI (1950): *Jui-chikusan-shinpo*, No. 47, 3 (in Japanese).
13) YAMAGIWA, S. (1950): *Ibid.*, No. 39, 6; No. 40, 7; No. 41, 10 (in Japanese).
14) YAMAGIWA, S. (1952): *Jap. J. vet. Sci.*, **14**, 523 (in Japanese).
15) YAMAGIWA, S., Y. FUJIMOTO, M. OHBAYASHI, T. ONO & K. OHSHIMA (1952): *Jap. J. vet. Res.*, **2**, 83 & 129.
16) YAMAGIWA, S. & K. OHSHIMA (1952): *Jap. J. vet. Sci.*, **14**, 411 (in Japanese).
17) YAMAGIWA, S., T. ONO & S. SUGANO (1951): *Ibid.*, **13**, 283 (in Japanese).
18) YASUDA, S., H. IMAOKA & T. IMADA (1941): *Oyo Juigaku Zasshi*, **14**, 25 (in Japanese).

Explanation of Plates

Plate I.

Fig. 1. Spleen puncture needle with inside needle. Sheathed steel needle 7.0 cm in length, 0.2 cm in outside diameter, and 0.1 cm in inside diameter.

Fig. 2. Spleen puncture needle with cylinder. Inside needle on the upper.

Plate II. Cells contained in spleen punctate. May-Giemsa staining × 900 oil immersion

Fig. 3 A. A λ cell on the lower side and a lymphocyte on the upper

Fig. 3 B. Three λ cells

Fig. 3 C. Three λ cells on the upper side and a segmented cell on the lower

Fig. 3 D. λ cells

Fig. 4. Plasma cell

Fig. 5. Lymphocytes

Figs. 6 A & 6 B. Monocytes

Fig. 6 C. Monocyte in the center and reticulum cells on the right and left side each, the left one phagocytizing an erythrocyte

Plate III.

Fig. 7 A. A reticulum cell on the upper side and a lymphocyte on the lower

Fig. 7 B. A reticulum cell

Fig. 7 C. Siderocyte in the normal spleen punctate

Fig. 7 D. Round-transformed cell with racket-like flat appearance

Fig. 7 E. Round-transformed cell containing fine granular foreign bodies in the cytoplasm

Fig. 7 F. Round-transformed cell with a kidney-bean-shaped nucleus

Figs. 7 G & 7 H. Round-transformed cell with a somewhat basophilic cytoplasm

Figs. 7 I, 7 J & 7 K. Different forms of macrophages

Fig. 8. Amitotic division

Plate IV. Proliferative change of λ cells in the spleen

Figs. 9 & 10. Small foci of proliferation of λ cells around a sheath artery. Horses Nos. 1 and 2, naturally infected cases in the field. Hematoxylin-eosin staining × 540

Figs. 11 & 12. Large foci of proliferation of λ cells around a small artery. Horses Nos. 17 and 24, experimentally infected cases. Hematoxylin-eosin staining × 540

Fig. 1

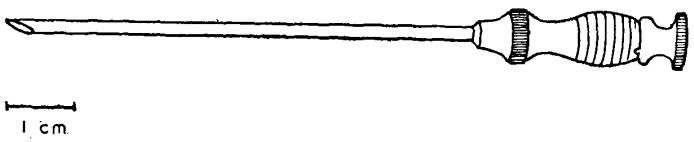
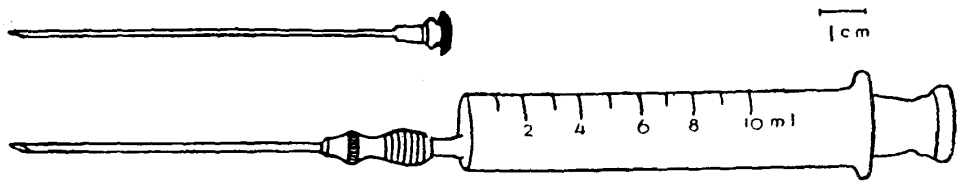
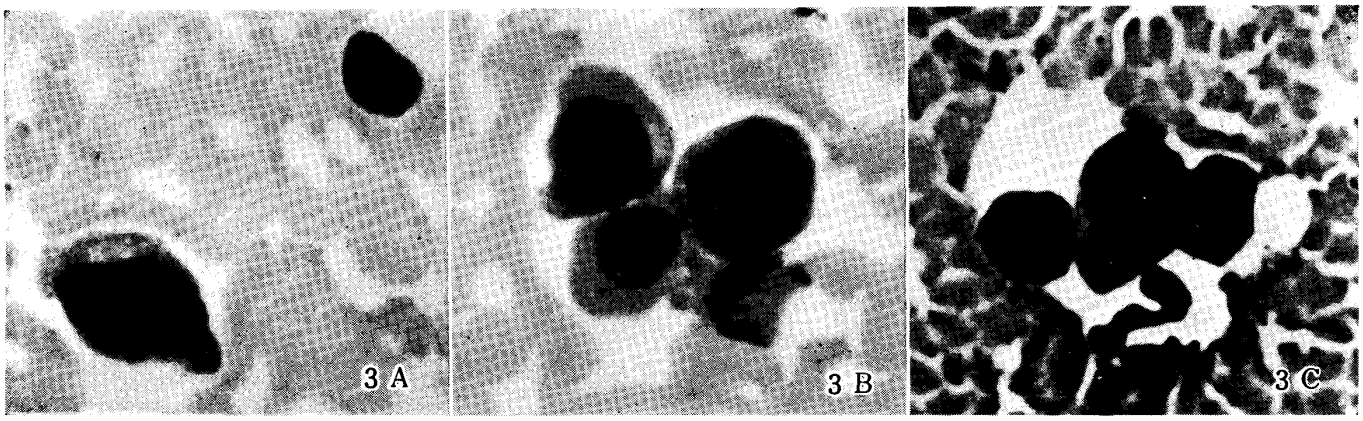
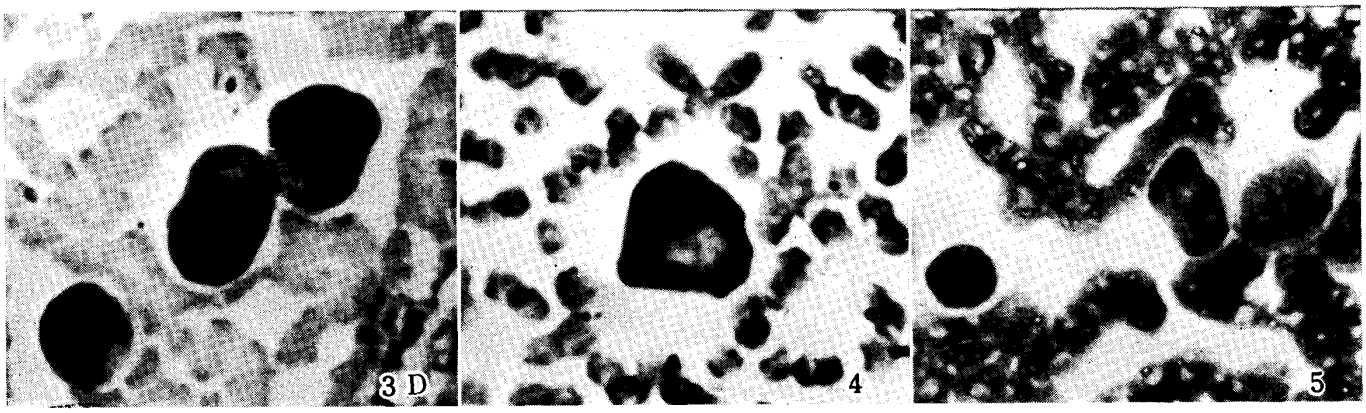
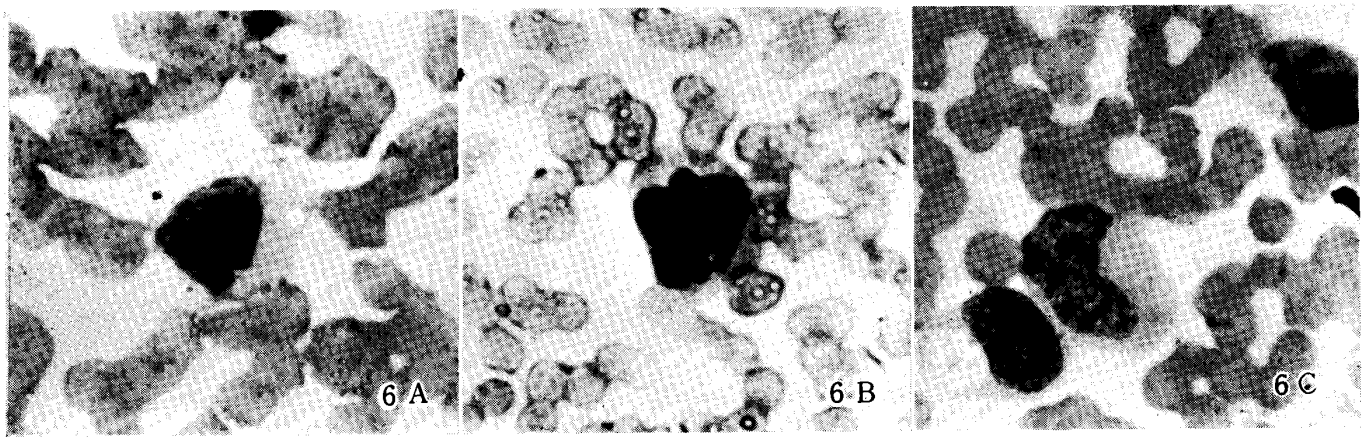


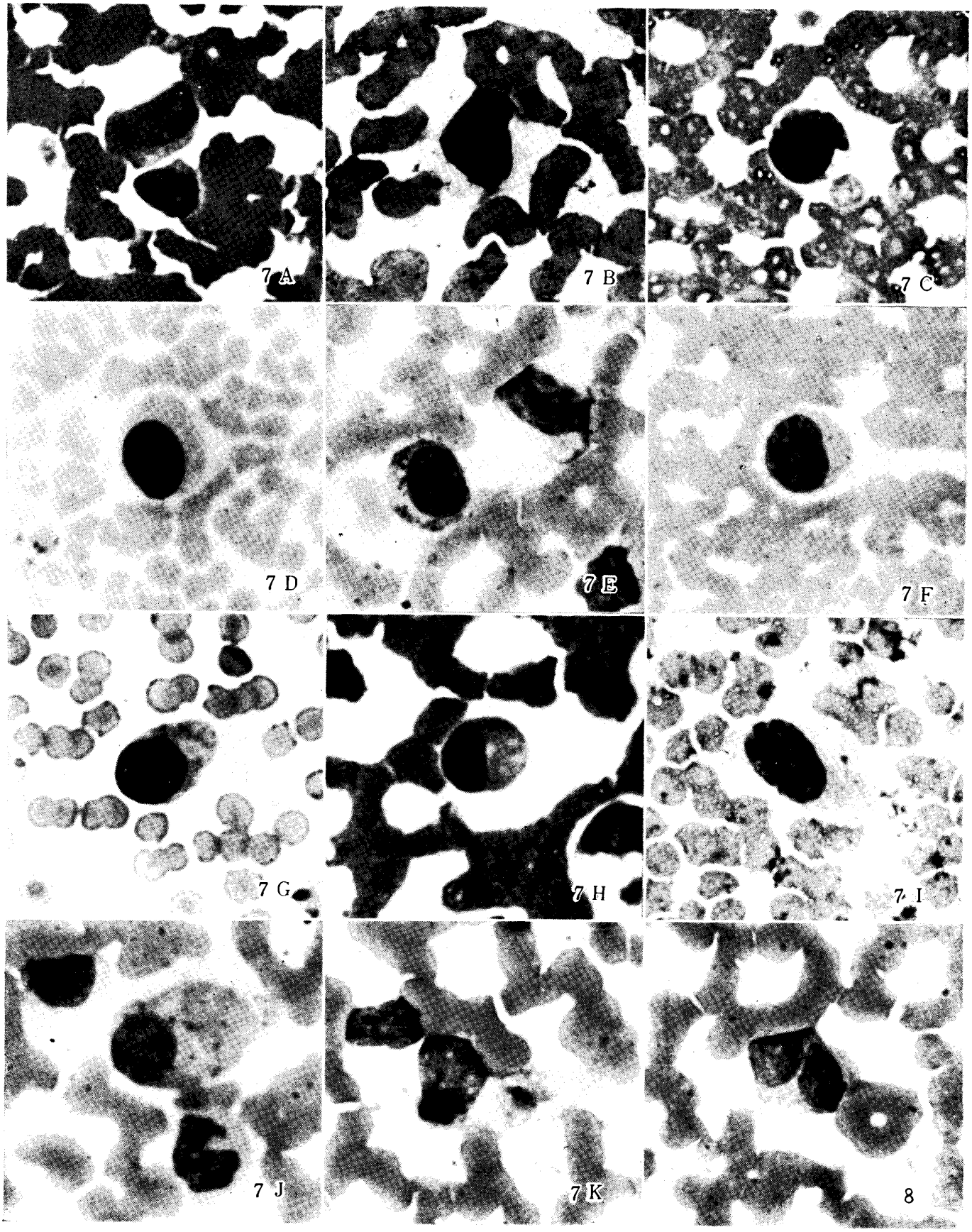
Fig. 2

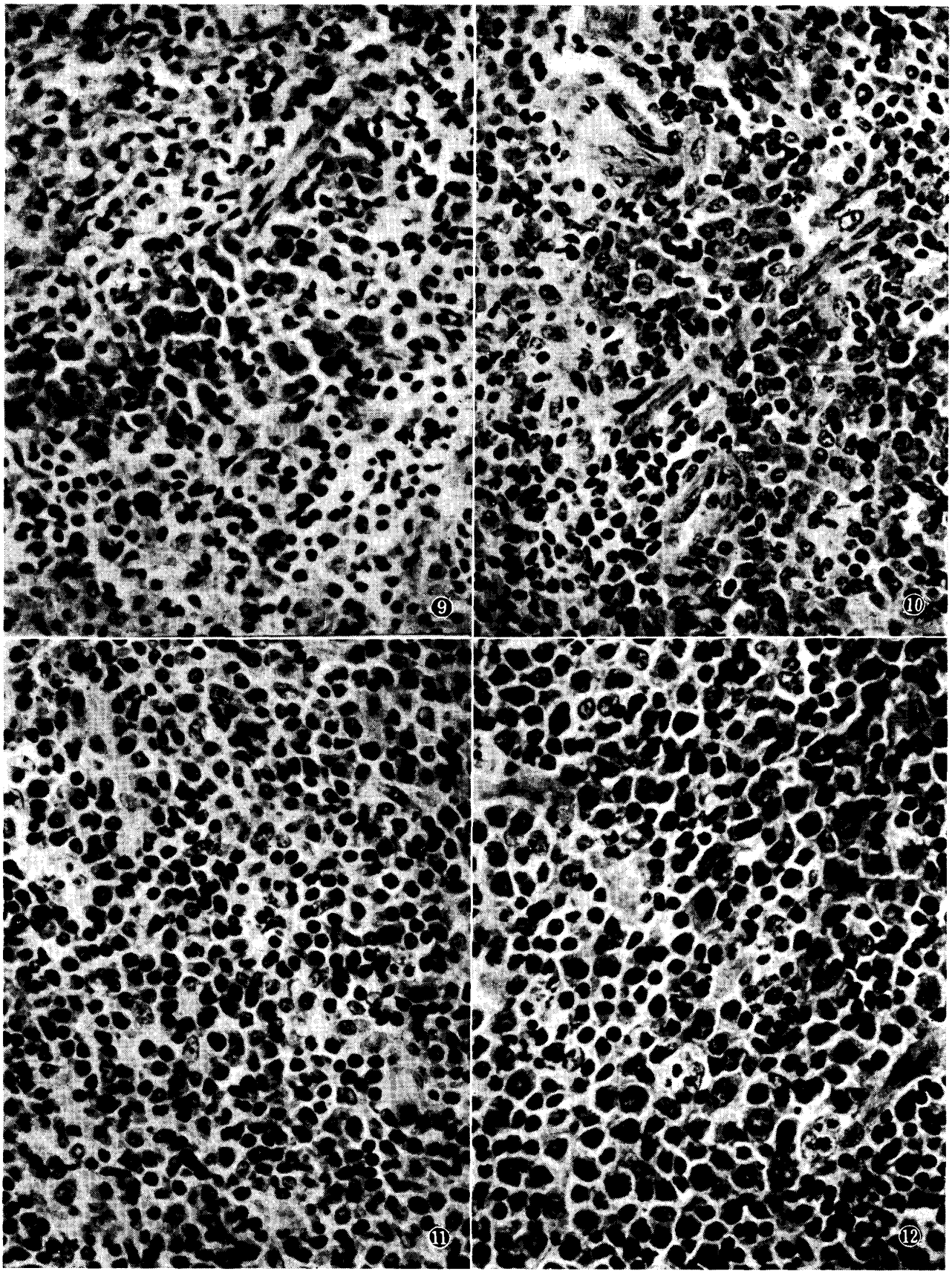



3 A
3 B
3 C


3 D
4
5


6 A
6 B
6 C


7 A
7 B
7 C
7 D
7 E
7 F
7 G
7 H
7 I
7 J
7 K
8


9
10
11
12