CYTOLOGICAL STUDIES ON LYMPH NODE IN EQUINE INFECTIOUS ANEMIA
I. CHARACTERS OF THE "*L-CELL*" AND ITS RELATIONS TO VISCERAL LESIONS

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Pathomorphological study on equine infectious anemia has been carried out in this laboratory during recent years. One sort of cell has drawn attention as a singular lesion; this cell element was recognized in such organs as lymph node, spleen and sometimes others. The cell, by hematoxylin-eosin staining, is seen to be as much as 2—3 times as large as a lymphocyte, its protoplasm basophilic, nucleus comparatively bright and chromatin substance poor. Nucleolus of the cell is stained basophilic and can be distinctly seen.

Prof. S. YAMAGIWA, the chief of the laboratory, and the present author named this cell element provisionally as the "L-cell"; some of the so-called "lymphoid cells" are identical to the "L-cell" and can be frequently discerned on histological sections of equine infectious anemia.

The present experiments were performed for the purpose of distinguishing the characters manifested by the "L-cell" and of contributing to the investigation of the nature of equine infectious anemia by clarifying the relation between the "L-cell" and visceral lesions.

MATERIALS

The materials examined were mainly from the slaughter house between the dates July 1, 1953 to April 1, 1954 and additionally some autopsied cases; cases which required examination and the control cases of non-equine infectious anemia were also examined. The study was carried out on these materials which included such cases of various ages from 6 months embryo to 19 year old adult. The number of examined cases numbered as many as 44.

As for the observations of lymph nodes, the lienal lymph node was primarily used as much as possible. In addition to this the hepatic, renal, iliac, mesenteric, bronchial and submaxillar lymph node as well as spleen, liver, kidney, lung, heart,

TABLE 1. *Subacute Type Cases Examined*

NO.	SEX	AGE (YEARS)	HOME	DATE OF SLAUGHTER	EXAMINED ORGANS
Z. 1	F	10	Ishikari	1/VII '53	Sl, S. H
Z. 9	F	11	Hiyama	1/VIII '53	"
Z. 13	M	16	Ishikari	14/VIII '53	"
Z. 15	G	4	"	11/IX '53	Sl, S, H, R

Note: See "Notes to Table 3".

TABLE 2. *Chronic Type Cases Examined*

NO.	SEX	AGE (YEARS)	HOME	DATE OF SLAUGHTER	EXAMINED ORGANS
Z. 2	G	8	Rumoi	4/VII '53	Sl, S, H
Z. 3	F	11	"	4/VII '53	"
Z. 4	"	10	Ishikari	6/VII '53	"
Z. 6	"	11	"	10/VII '53	"
Z. 8	"	13	"	30/VII '53	"
Z. 10	"	8	Hiyama	1/VIII '53	"
Z. 11	"	5	Ishikari	4/VIII '53	"
Z. 12	"	8	"	12/VIII '53	"
Z. 19*	M	4	"	24/X '53	Sl, Rl, S, H, R
Z. 20*	F	7	Abashiri	"	"
Z. 21	"	1/2 (Embryo)	?	27/X '53	Sl, S, H
Z. 22*	G	11	Ishikari	6/XI '53	Sl, Rl, S, H, R
Z. 24	F	17	"	16/XI '53	"
Z. 25	"	6	Rumoi	"	"
Z. 26	"	17	Shiribeshi	"	Sl, Rl, S, H R, C, L, I
Z. 30	M	2	Hidaka	26/XII '53	Sl, Hl, Mel, Ml, S, H, R, C, L, T, A
Z. 31	F	18	Rumoi	"	Sl, S, H, R, C, A
Z. 33	?	?	?	6/II '54	Sl, Hl, Rl, S, H, R, A
Z. 34	?	?	?	"	Sl, S
Z. 35	G	16	Ishikari	8/III '54	Sl, Hl, L, Ml, S, H
Z. 36	F	9	"	10/III '54	Sl, Hl, Rl, S, H, R
Z. 37	"	18	"	11/III '54	Sl, Hl, Ll, Ml, S, H. R, C, L, A, M, To
E. 998*	M	18	Hidaka	10/IX '54	Sl, Hl, S, H, R, C, L, P, T
E. 1374*	F	2	Ishikari	25/I '54	Sl, Hl, Rl, S, H, R
E. 1382	"	13	"	25/II '54	Sl, Hl, Rl, Il, S, H, R, M, O, I
3532	"	13	"	13/I '54 (Death)	Sl, Rl, S, H, R
3559	"	2	"	2/IV '54	"

Notes: *: Lesions are still more active. See "Notes to Table 3".

suprarenal gland, testicle, ovary, submaxillar gland, tonsil and thymus were simultaneously investigated.

Throughout the histopathological examination, these cases were divided into two types of equine infectious anemia and the control; 4 cases were the subacute type (Table 1), 27 the chronic type (Table 2) and non-equine infectious anemia (Table 3) (the name of type was initiated by YAMAGIWA et al).

TABLE 3. *Non Infectious Anemia Cases Examined*

NO.	SEX	AGE (YEARS)	HOME	DATE OF SLAUGHTER	EXAMINED ORGANS
Z. 5	M	1	Ishikari	6/VII '53	Sl, S, H
Z. 7	F	15	Abashiri	21/VII '53	"
Z. 14	"	6	"	14/VIII '53	"
Z. 16	"	18	Hidaka	11/IX '53	"
Z. 17	"	19	"	"	"
Z. 18	G	15	Ishikari	"	"
Z. 23	F	"	Abashiri	7/XI '53	Sl, Rl, S, H, R
Z. 27	"	13	Hidaka	26/XI '53	Sl, S, H
Z. 28	M	2/3(Embryo)	"	"	Sl, Hl, S, H, R
Z. 29	F	18	"	"	Sl, Rl, S, H, R
Z. 32	M	2	Tokachi	18/I '54	Sl, S, H
Z. 38	F	3/4(Embryo)	Ishikari	11/III '54	Sl, Hl, Rl, Il, Ml, S, H, R, C, L, A, Th
Z. 39	G	16	"	1/IV '54	Sl, S, H

Notes: Sex—M: Male, F: Female, G: Gelding.
Examined Organs—H: Liver, S: Spleen, R: Kidney, C: Heart, L: Lung, A: Suprarenal Gland, O: Ovary, T: Testicle, P: Pancreas, To: Tonsil, Th: Thymus, M: Submaxillar Gland, I: Intestine, Hl: Hepatic Lymph Node, Sl: Lienal Lymph Node, Rl: Renal Lymph Node, Ll: Bronchial Lymph Node, Il: Iliac Lymph Node, Mel: Mesentric Lymph Node, Ml: Submaxillar Lymph Node.

METHOD

For the histological examination, the materials were fixed with CARNOY's and HELLY's solution and paraffin section was made which was further stained with hematoxylin-eosin and UNNA-PAPPENHEIM's pyronin-methylgreen. FEULGEN reaction was conducted with some sections.

For the cytological studies, touch preparation of the lymph nodes as well as other organs and the lymph smear were made. GIEMSA and MAY-GIEMSA staining were adopted. The preparation, after the non-dried fixation by KUNII's P.E.F.C. solution, were stained with MANN's methylblue-eosin solution.

At the time of the supravital staining, the lymph was used by reason of the

remarkable dilatation of lymph vessels in lymph node of usual equine infectious anemia cases; the lymph was obtained by direct absorbing with injection syringe from the dilated lymph vessel, but if the lymph vessel was not dilated, the tissue liquid on cut surface of lymph node was prepared. The phase difference microscope (immersion, dark) was used for the morphological observation of the living cell as well as for the supravital staining.

RESULTS

1. Characters of the "L-cell"

Before describing the character of the "L-cell", the ordinary occurrence of the cell and its increase must be briefly explained; the details of distribution will be described afterwards. A few of the cells can be recognized in medullary cord and follicle center of the lymph node section in normal condition but the cell is markedly increased in equine infectious anemia, and in some extreme cases, the lymph sinuses are filled with "L-cells". In the MALPIGHI's corpuscles and the red pulp of spleen, a few "L-cells" are recognized, too.

By hematoxylin-eosin staining, as noted above, the protoplasm is stained basophilic, having a thick nuclear membrane; the nucleus is poor in chromatin and is found to two or several nucleoli stained basophilic. The cell is two or three times larger than the lmphocyte with sizes of over 15~16 μ and sometimes 30 μ in diameter (Figs. 1 and 2).

In staining by UNNA-PAPPENHEIM's solution, the protoplasm and the nucleoli are pyroninophilic, and the FEULGEN reaction of the nucleolus is negative.

By MAY-GIEMSA staining, protoplasm and nucleoli are stained basophilic, and nucleus is round, oval or sometimes kidney-shaped. Some of the protoplasm shows a bright spot in the portion in contacted with the nucleus. The nucleolus as well as the protoplasm granules is recognized in the well-stained preparation (Fig. 3).

After non-dried fixation, the "L-cell" stained by methylblue-eosin shows red nucleoli clearly which take various shapes such as roundish, triangular or long oval etc. and also various sizes (Fig. 4).

Observation under the warm phase microscope with supravital staining discloses no neutral-red granules and a few janusgreen granules in the protoplasm of "L-cell". It discloses within the protoplasm of cell the red staining of only vacuoles which are considered to be abortive in form. The figure under the phase microscope show the syrupy condition of the "L-cell" protoplasm, thickened nuclear membrane and the yellowish orange coloured nucleoli separated from nuclear membrane. Carbon particle phagocytosis of the cell is not observable (Figs. 5~11).

2. The "L-cell" in Visceral Lesions

Regarding to the relation between the "L-cell" and visceral lesions as shown in the above tables, the materials subjected to the author's examination were 4 cases of the subacute type and 27 cases of the chronic type. In describing the results, generalizations will be stated for each organ since the degree of appea-

rances of "L-cell" in various organs shows no essential difference. It is the tendency to consider that the lymph node changes are different depending upon the area where they belong, but as the lymph nodes consist of the same tissue named lymphoreticular tissue, reactions against the agents inflicted upon the living body will surely appear as systematic lesions. The foregoing statement is plain enough according to the detailed studies on the lymphoreticular tissue made by ONO in 1940. The present author in his the histological study on lymph nodes in equine infectious anemia also confirmed that no essential differences occur in accordance with the area to which they belong. Thus no individual illustration is necessary in this description, generalizations are in order.

a) *Lymph nodes.* The observation was conducted mainly on lienal lymph nodes. The "L-cell" increases in medullary cord primarily both from the middle zone and to the center of follicles. Generally the increase is not very severe in the chronic type and some "L-cells" are recognized in the medullary and intermediate sinuses in contamination with several lymphocytes and reticular cells. In the chronic type, which is still more active (Z 19, Z 20, Z 22, E 998, E 1374), and the subacute type, the "L-cells" severely increases in the medullary cord becoming more numerous than the originally existing lymphocytes; all the marginal lymph sinuses as well as the medullary and intermediate sinuses are frequently filled up with the increased "L-cells" (Fig. 1).

b) *Spleen.* The increase of the "L-cell" in the MALPIGHI's corpuscles is similar to the appearance in the lymph follicle. In the splenic pulp of equine infectious anemia, the "L-cell" increases uniformly having some relation to the island-like or map-like cell-increased foci described by YAMAGIWA et al., however, it is noticed that the cell increases occur not only in such foci but diffusely throughout the whole red pulp. In YAMAGIWA's cell-increased foci, it is observed that plasma cells are conspicuous peritubularly; they are considered to be differentiated from adventitial element. Sometimes the dilated lienal sinus contained a few "L-cells", however, the "L-cell" is deprived of almost all its pyroninophilic character which is the distinctive point in the appearances in the lymph sinus.

c) *Liver.* The "L-cell" is not noticeable in the so-called cell foci of the liver in equine infectious anemia. Of the chronic type, 11 cases (Z 2, Z 6, Z 11, Z 12, Z 20, Z 30, Z 35, Z 36, E 1382, 3532, 3559) have few "L-cells", 9 cases (Z 4, Z 8, Z 10, Z 19, Z 21, Z 24, Z 26, Z 31, E 998) have a few, and in 5 cases (Z 3, Z 22, Z 25, Z 33, Z 37) the "L-cell" is recognized sporadically accumulated two to several in the central veins and intralobular capillaries. In only one case (E 1374), was there a dilatation of the intralobular capillaries in which many nodule-like foci of the "L-cell" were recognized. This appearance is similar to that of the subacute type in which many more increased typical "L-cells" and a few reticular cells are noticed (Fig. 2).

The cell infiltrations in GLISSON's sheath are mainly lymphocytic and contained several plasma cells.

d) *Kidney and others.* In the interstitial lymphocytic infiltration which is

recognized in chronic type equine infectious anemia, "L-cells" can never be seen. In the kidney of subacute type (Z 15), the "L-cells" are sometimes recognized sporadically but usually intracapillarly, accompanying the dilatation of the capillaries, numbering two to several. Also sometimes they are recognized sporadically in extracapillaries. Against these appearances only 7 cases of the chronic type (Z 20, Z 22, Z 25, Z 33, Z 37, E 1374, 3532) have a few "L-cells" intracapillarly, except one case (E 1374) which had become severe similar to the subacute type. It is noticed that the "L-cells" which appeared are considerably weaker in their pyroninophilic character.

Investigations of the "L-cells" in other organs were carried out on the chronic type and a few "L-cells" were recognized intracapillarly in lungs (Z 30, Z 37), suprarenal glands (Z 30, Z 33, Z 37) and testicle (Z 30).

DISCUSSION

In this investigations, the "L-cell" shows a new independent character throughout the morphological and histochemical studies as well as a new appearance in the phase microscope. These facts suggest to the writer that the "L-cell" may be distinguished from other cells even if it should be a part of a stage of a cell differentiation. That is, it is characterized by comparatively large, basophilic and syrupy protoplasm, pyroninophilic nucleolus, thickened nuclear membrane, janusgreenophilic character and non-phagocytosis to carbon particles by supravital staining, etc.

The author identified this "L-cell" as the cell recognized by AMANO et al. and UNNO in the popliteal lymph nodes of rabbits to which solesubcutis was experimentally injected with hen blood. AMANO et al. called this cell the "lymphogonia" through some cytological consideration and concluded that the cell transformed from the reticular cell. At the present time, however, several problems are left to be discussed on the lymphogonia-theory and, until the origin and future of the cell can be clarified, the author has also to make the reservation that it may be permissible to call the "L-cell" by the name "lymphogonia".

It is needful, therefore, in this report, to describe the differences between the "L-cell" and other cells which are likely to be confused with the "L-cell".

Plasma cell. This sort of cell possesses pyroninophilic, basophilic and syrupy protoplasm, but the nucleus abounds in chromatin and is rather small; consequently the protoplasm is comparatively large. The nuclear membrane is papillary thickened inside the nucleus, showing so-called axial structure under the phase microscope, and the nucleoli which

show the pyroninophilic character are contained in some part of the thickened membrane; it is not difficult to identify the plasma cell (Fig. 12).

Large lymphocyte is stained slightly basophilic by the GIEMSA staining; nucleoli are not pyroninophilic, and they are obscurely recognized but observable as independent spots under the phase microscope.

Reticular cell is sometimes rather indistinguishable from the "L-cell"; the protoplasm is hardly stained by the GIEMSA staining; nucleolus has no pyroninophilic character; nuclear membrane is thin, and it shows energetic protoplasm motion and active phagocytosis under the warm microscope (Fig. 13).

Monocyte is the most indistinguishable by the GIEMSA stainig. Most of the monocytes possess a multilobular or dented nucleus having the thinner membrane than that of the "L-cell"; the nucleoli are not very clear, and the protoplasm granules exist partially, therefore it is not always difficult to distinguish. The nucleoli of monocyte are not pyroninophilic by the pyronin-methylgreen staining, and the most essential differences are observed by the supravital staining, viz., so-called rosette formation of neutral red and the dull stretching of the protoplasm processes (Figs. 14~16).

Pertaining to the relation visceral lesions with the "L-cell", it is obvious that the "L-cell" increases in the medullary cord of the lymph node. According to YAMAGIWA and YAMAGIWA et al., the lesions in equine infectious anemia are divided into four types; viz., the acute, subacute, chronic and the relapsed type. However, the acute and relapsed type were not observed in this study. However, those type are not always necessary for "L-cell" studies. In the acute type the main lesions show parenchymatous degeneration and reticular system activities, and in the relapsed type the chronic lesions are associated with the acute or subacute lesions reacting against some factors. In other words, the increase of the "L-cells" is the severest in the subacute type. It is noted above that the typical "L-cell" increases in the medullary cord of the lymph nodes becoming predominant over the originally existing lymphocytes and frequently filling up the lymph sinus in the subacute type as well as in some cases of the chronic type. This is due to nothing but the fact that the rapidly increased "L-cells" in the medullary cord move out into the lymph sinus.

The increase of the "L-cell" in the spleen is rather less than in the lymph node; the typical "L-cell" in the lienal sinus is not very

frequently seen. It is considered that the "L-cells" increase slightly in red pulp and it do not move out into the lienal sinus.

According to the previous description, it is distinct that the appearances of the "L-cells" in the liver, kidney and others are not only paralleled with the increase of "L-cells" in the lymph node, but also that these cells are almost all in the blood vessels and only a few of them escape into the interstitium.

It seems to assume that the "L-cells" which increased mainly in the medullary cord of the lymph node move out into the lymph sinus and then into the circulation system through the lymph vessels and finally appear in the visceral lesions as a result of circulation in the blood. This also a reasonable assumption because the most of the "L-cells" in the visceral lesions, except the lymph nodes, decrease in pyroninophilic character and atrophy. But there are some problems as to the mechanism of the movement of the "L-cell" out of the lymph node similar to the problem of HORII et al. in their study of the manner in which the monocytes in the lymph nodes escape through the walls of the capillaries. In the present studies the "L-cell" is recognized in the efferent lymph vessel of the lymph node sections and the lymph smear from the efferent, therefore it might not be wrong to consider that most of them are carried away by the blood circulating through the lymph vessel.

The following two questions have to be solved: one of them is, "if the cells are carried away together with the circulating blood, can they be recognized in the peripheral blood ?"; and the other is: "if they are recognized in the peripheral blood, what name has been given to the "L-cell" by the past investigators of the clinical hematology in equine infectious anemia ?" The author has to answer "yes" to the former question. As the present study is not primarily a hematological study, detailed data will be reported with the future observation, but now, it may be said that "L-cells" could be recognized in peripheral blood in our several cases. To the latter question, it may be answered that the monocyte has been noticed which was brought up to discussion in the past clinical hematology in equine infectious anemia. MIURA et al. (1950) in their *"Clinical Hematology of Equine Infectious Anemia"* state, *"In a group of the cells named monocyte, the cells are not the same in their physiological function, and the cells having different characters are ···."* This is nothing but to admit that there has been no cytological research done on the monocytes with a few exceptions. In regard to these,

DE KOCK (1925) pointed out the lack of clear differentiation between the lymphocytes and some of the monocytes in his studies in equine infectious anemia. And recently AMANO et al. cautioned against the confusion of the monocyte and their "lymphogonia" in the GIEMSA staining.

Secondarily the explanation of the origin and future of the "L-cells" has to be presented. With regard to the lymphogonias, AMANO et al. believed that they were differentiated from the reticular cells. The author has decided from the histological point of view that the "L-cells" would originate in the lymphoreticular tissue. And it is presumed that in the future the "L-cell" may be found to be a sort of lymphocyte.

Supporting this view, in regard to the cytochemical appearances, the pyroninophilic nucleolus and its negative FEULGEN reaction do show presumably the presence of ribonucleic acid, but the final determination of its existence is proved by digestion using ribonuclease. This shall be cleared with the investigation of the function of the "L-cell".

SUMMARY

Cytological studies were made on the "L-cell" which appeared on the section especially of the lymph node in equine infectious anemia that the cell is a sort of the lymphoid cells. As a result, the "L-cell" is identified with the lymphogonia reported by AMANO et al. in 1951. It is considered that the "L-cell" is originated from the lymphoreticular tissue, and it is assumed that it will prove to be a sort of lymphocyte.

About the relation between the visceral lesion and the cell, one of the most important feature is the neoplastic increase of the "L-cell" in the lymphoreticular tissue reaction after some sort of irritations (e.g. the virus of equine infectious anemia or its production) with the possibility of having some purposes; and it is considered that those increased "L-cells" are carried away through the circulating system into whole body.

Yet, regarding the "L-cell" many problems remain for future consideration.

At the conclusion of this paper, the author, wishes to express his gratitude to Prof. S. YAMAGIWA for his kind direction and review, and to Assist. Prof. S. AMANO and Dr. M. HANAOKA, University of Kyoto, for their kind instruction in regard to the "lymphogonia".

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EXPLANATION OF THE PLATE

Plate I

Fig. 1. Arrows show "L-cells" in medullary cord of lymph node. H.-E. staining, × 530.

Fig. 2. Arrows show "L-cells" in central vein of liver. H.-E. staining, × 530.

Fig. 3. An "L-cell" in the center of the figure. The others are lymphocytes. *May-Giemsa* staining, × 1300.

Fig. 4. L: An "L-cell". M: Mitosis. Mo: A monocyte. The others are lymphocytes. *Mann* staining after non-dried fixation, × 1300.

Figs. 5~6. Various "L-cells". Phase difference figure, × 1400.

Plate II

Figs. 7~9. Various "L-cells". Phase difference figure, × 1400.

Fig. 10. An "L-cell" with a vacuole. Phase difference figure, × 1400.

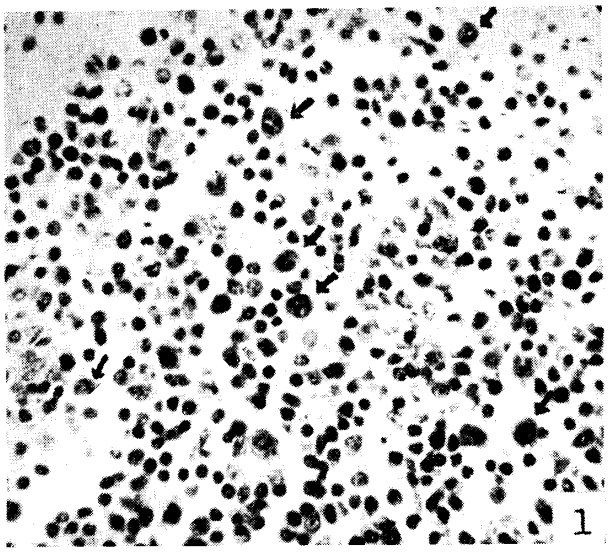
Fig. 11. Mitosis of a cell considered to be an "L-cell". Phase difference figure, × 1400.

Fig. 12. A plasma cell; the nuclear membrane is papillary thickened. Phase difference figure, × 1400.

Plate III

Fig. 13. A reticular cell; the nuclear membrane is not clear. Phase difference figure, × 1400.

Figs. 14~16. Monocytes; dented nucleus and partially existed protoplasm granules are distinct. Phase difference figure, × 1400.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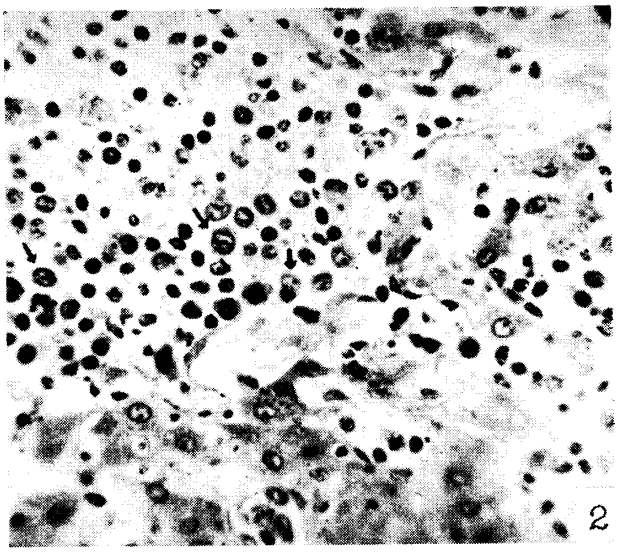
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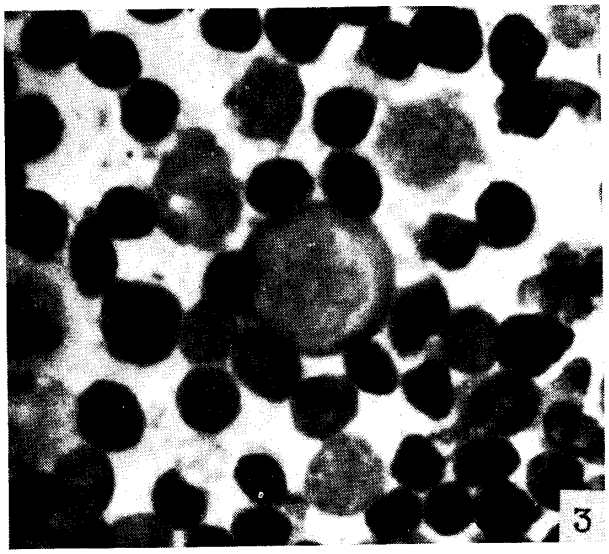
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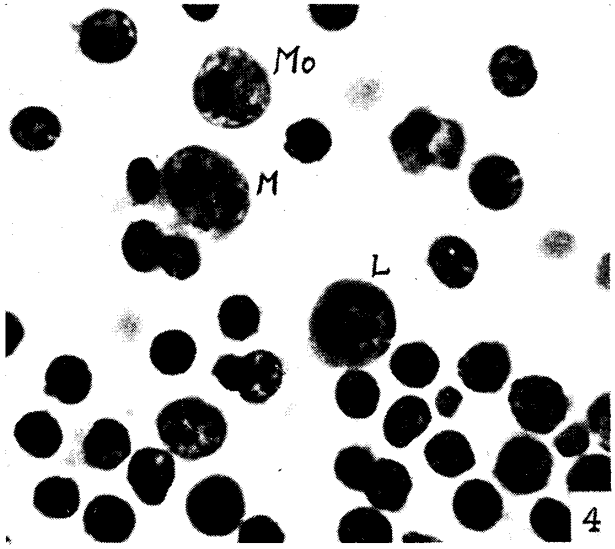

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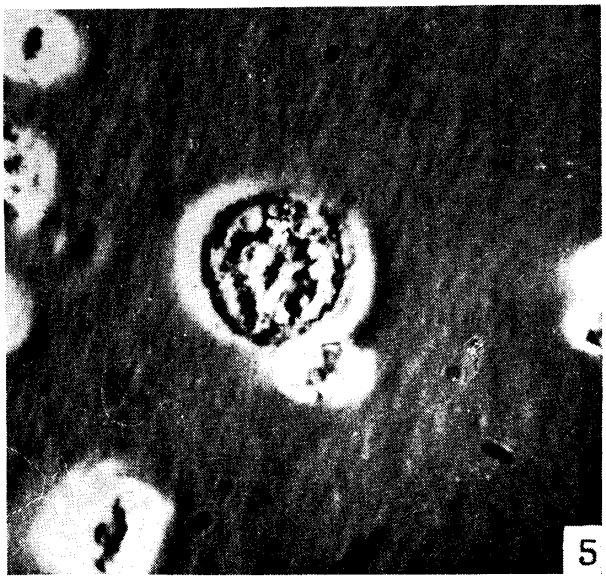
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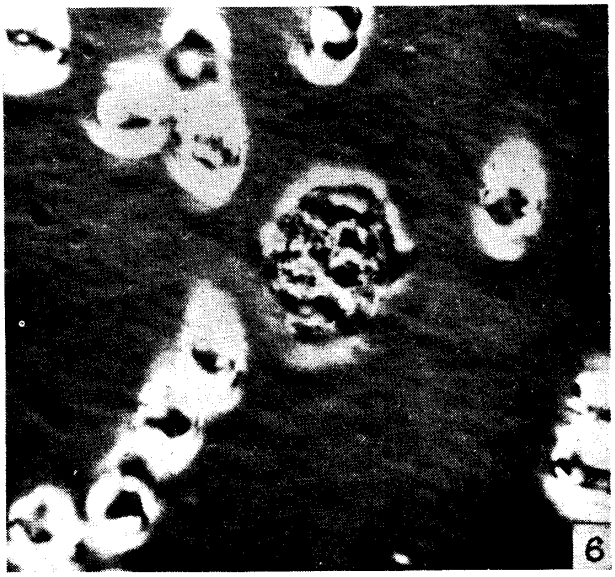
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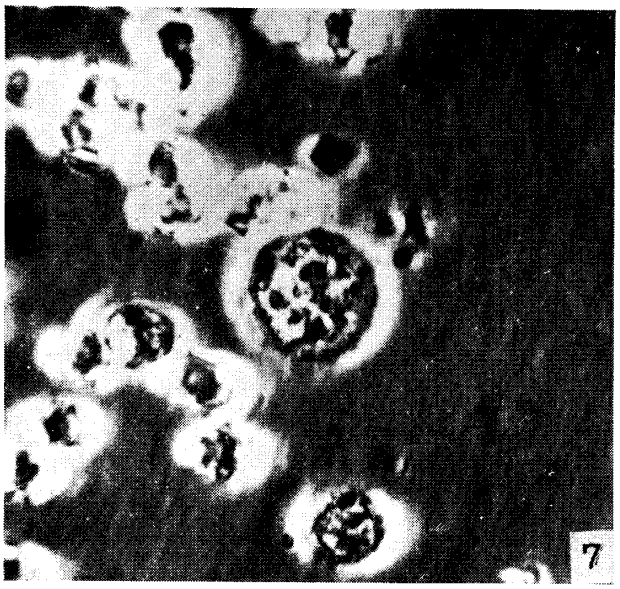
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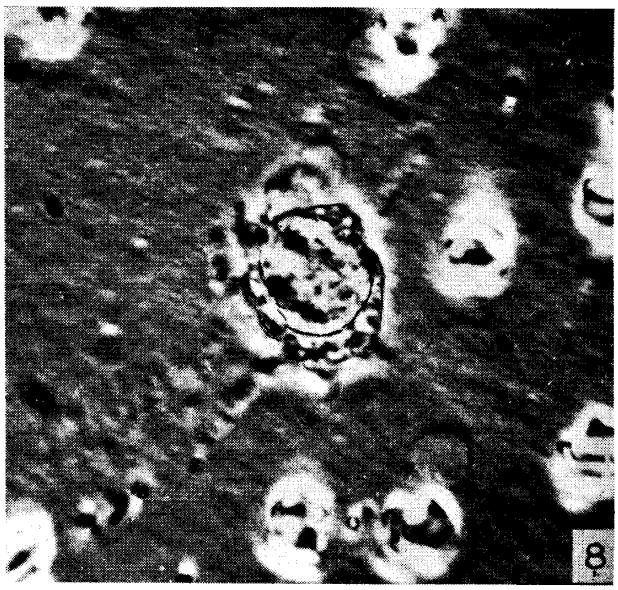
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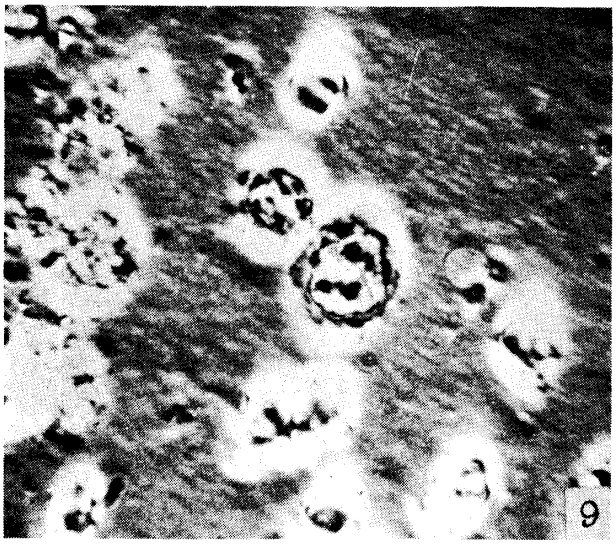
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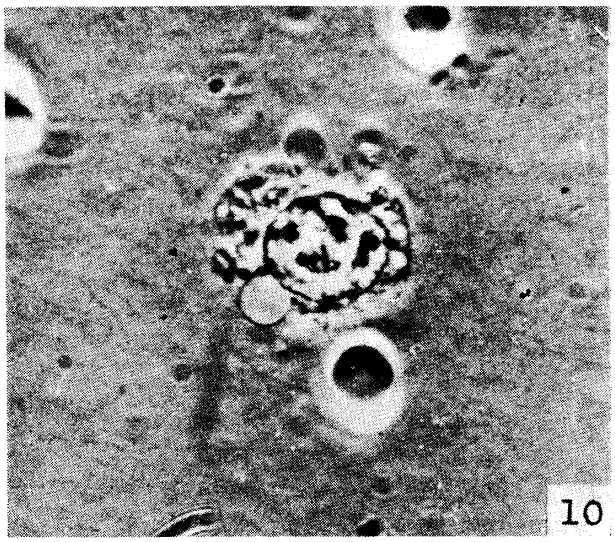
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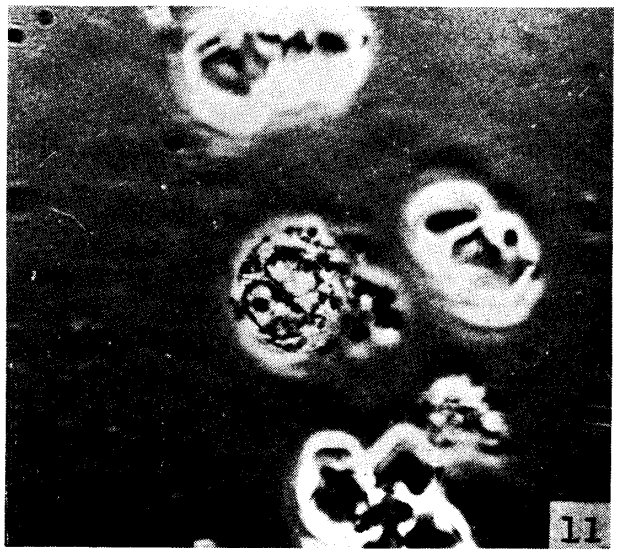
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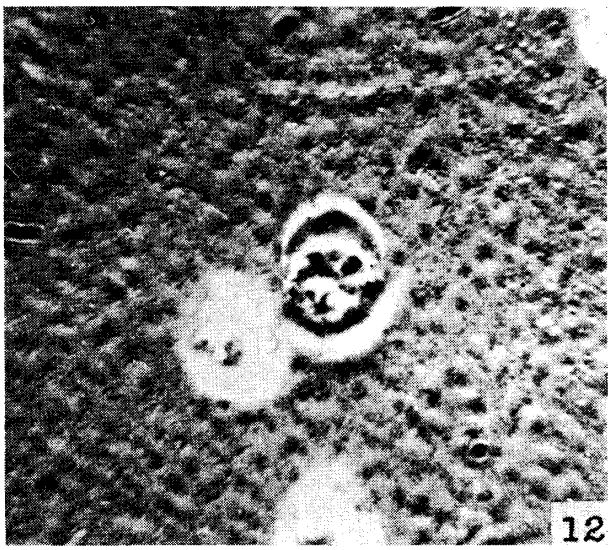
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OHSHIMA, K.