# STUDIES ON AVIAN VISCERAL LYMPHOMATOSIS I. THE INCREASED INCIDENSE AMONG CHICKEN FLOCKS AND PATHOLOGIC PICTURES

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t was certainly not in this country until the Korean (1950—1952) that cases of the overt avian leukosis certed in the field in any appreci able numbers. After War, during several years, the number of chicken cks increased rapidly and many poultry farms were ablished with a few imported breads. From that ne, the only type of leukosis observed in the field of is district was what is now known as visceral lymphnatosis, and inconceivably no other types than visceral rms have seemingly appeared in any appreciable numers up to this day. At that time this condition was ridely believed by poultry-men to be caused by feeding neat or fish scrap, and was known popularly as "fish crap liver" or "big liver disease."

According to the survey by Winton (1951-1954), about 60 percent of total mortality of chickens for the period from 1932 to 1947 in the U.S. was caused of avian leukosis complex; lymphomatosis was responsible for 30 percent (one-half of total mortality), and the visceral form caused two-thirds of lymphomatosis. An another estimate of the importance of lymphomatosis was made in results of the New York Random Sample Test. in which many poultry breeders from different sections of the U.S. entered their stock from 1950 to 1954. The yearly average total mortality of the hear, which were hatched and reared from eggs received at the test, was 34 percent. Lymphomatosis was responsible for 13.4 percent; the visceral form caused three-fourths of this mortality. If that death rate were applied to the total number of chickens on farms, it could be estimated that

in 1954 more than 59 million mature chickens died of it. Highly instructive epizootiologic data have also been presented by Blaxland (1956) and Chubb and Gordon (1957).

There hardly can be any doubt that under the conditions of modern intensive poultry production, visceral lymphomatosis represents by far the most common form of avian neoplasia and became a definite economic hazard. This form of the disease widely spread in flocks of chicken throughout this country and heavy losses from it was estimated on many farms of this district in the 1960's, but no data concerning the spontaneous incidence and pathologic pictures of avian leukosis in chicken flocks in this country can be found in the literature.

To determine, therefore, whether there is any actual increase in the incidence of avian visceral lymphomatosis in the field, an analysis was made of the postmortem and histopathologic findings.

### MATERIALS AND METHODS

Out of 3,5000 chickens examined during the period of 1961 to 1963 at the fowl retailers in Chunchon district and clinical laboratories 266 cases of visceral form of lymphomatosis were collected. The specimens secured from the fowl retailers were brought to the laboratory for examination and recording of details.

Portions of 42 specimens which were relatively fresh and unfrosted were fixed in 10% formalia for bis-dword examination. Hematoxylin and cosin stain was used one tinely for paraffin sections.

# RESULTS AND DISCUSSION

Incildence of Visceral Lymphomatosis During the Study:

For the period 1961 through 1963, the postmortem records covered a total of 3,500 chickens, of which 266 (7.6 percent) showed visceral form of lymphomatosis. Chicks under 8 weeks of age were not included in this analysis. The data for the three-year period have been arranged in the following tables. Table I shows the total incidence of visceral lymphomatosis found in all chickens autopsied during the study, and compiled without regard to breeds in order of years.

Table 1. Total Incidence of Visceral Lymphomatosis During the Study

Year	Number Autopsied	Cases of Visceral form	Percent of Visceral form		
1961	1,650	154	9.3		
1962*	1,100	75	6.8		
1963*	750	50	6.7		
Total	3,500	266	7.6		

 Total numbers of chicken flocks in Chunchon district decreased for the reason of feed supply difficulty year by year.

Many investigators have given data on the incidence of avian leukosis in various breeds (e.g. Gross, Hult, Cole and Bruckner, Sturkie, Bryant and Johnson, Water and Prickett, Hay, Quortrup and Shillinger, Hoffman and Stover, Olson and Bullis \*), in broiler and replacement birds (Benton et al., 1957) or male and female chickens (Burmester, 1945) since 1940's. Among them the data for the twelve years 1934 through 1945 by Davis et al. (1947) in Indiana showed total 13,669 chickens autopsied, of which 4,750 were affected with some form of leukosis. Above all, in 2,013 White Leghorns autopsied, 981 (48.7 percent) showed lesions, and in 6,555 of Barred and White Plymouth Rocks autopsied, 2,595 (39 percent) showed lesions respectively.

Two transmission experiments by Davis et al. (1947, 1949) also show that the incidence of visceral lymphomatosis was increased by inoculation with cell-containing material from lymphomatous field cases, as 9.6 and 16.2 percent in control against 38.8 and 43 percent in inoculated birds, respectively.

It is regrettable that no distinct data on the incider in various breeds of chickens were made in this stude because all chickens raised and autopsied in this are were unfortunately not given exact lines of breeds. The autopsied chickens represented largely by Single Comwhite Leghorns, mixed breeds or other breeds whice were in too small number to have any statistical significance.

In contrast to the surveys of earlier investigators, the percentages of visceral form occurred here were apparently less that of foerign. This figure supposedly was caused by less dense population of chickens raised here, and implies that this form of the disease is more frequently transmitted from imported foreign breeds of chickens. For this reason, avian leukosis did not appear in native Asiatic breeds of chickens in the earlier days in this country.

# Gross Findings of Affected Organs:

Samples of liver, spleen, ovary, and in some cases intestine from 266 positive cases of visceral lymphomatosis were studied grossly. In the postmortem findings, individual chicken showed lesions in the miscellaneous visceral organs. In a few cases, the so-called "big-liver" was constitute almost one-half the total weight of the chicken. In such cases, the spleen and kidney were also, involved. Ascites frequently was found in the cases. Table 2 shows the comparison of gross lesion distribution in various organs. Most often times the liver alone was affected, but a little less than one-half of livers were affected in various combination with different organs.

Table 2. Comparative Distribution of Gross Lesions in Various Organs of Visceral Lymphomatosis Cases

D) inphomatoon Cases						
Distributed Organs	Number of Affected Organs	percent of Affected Organs				
Total Liver Cases: Liver alone	243: 129	91.4: 48.5(53.1 of total affected liver)				
L+Sp+Kid +Ov+Int. L+Sp. L+Ov. L+Kid. L+Int. L+Sp+Ov. L+Sp+Kid. L+Ov+Kid. L+Ov+Int.	3 46 18 19 3 12 4 6 3	1.1(1.2 " ) 17.3 6.8 7.2 1.1 4.5 1.5 2.3 1.1				

<sup>\*</sup> Citedby Davis et al. (1937)

Other than Liver Cases:	23:	8.6;
Spleen alone	4 /	1.5
Ovary alone	8	3.0
Intestine alone	3 (11)	1.1
Sp-Ov.	3 3	1.1
Kid-Ov.	2	0.8
Int-Ov.	3 ;	1.1

<sup>\*</sup> L=Liver. Sp=Spleen, Kid=Kidney, Ov=Ovary, Int=Intestine.

In such combination, liver plus spleen was much commoner (more than one-thirds); liver plus kidney or liver plus ovary followed next with the same rate, respectively; trinal organs affected with livers were occurred in a few numbers. The cases in which all five organs were affected were observed in only three incidences. In the cases of normal livers with other affected organs, only ovary, spleen and intestine alone were occurred in a few numbers respectively; and combination with other organs were occurred in seldom numbers with no significance.

probably no other disease of chickens preents a greater variety of gross pathologic pictures than visceral lymphomatosis. According to Jungherr (1952), Burmester et al. (1947), Horiuchi et al. (1958) and other workers, there is really no organ of the body which does not show gross

alterations. A few organs are principally affected, such as liver, kidney, spleen and ovary; and bone marrow, thymus and bursa of Fabricius are also frequently invo lved; but heart, lungs, pancreas, mesentery and skin are seldom affected. Among such organs the liver showed gross lesions more frequently, but incidence was very rare that the organs other than liver alone showed gross manifestations in this study. And then in the field postmortem cases, livers were the most outstanding organ for interpretation of gross lymphomatous changes unlike as specimen.

In all cases of visceral lymphomatosis of two transmission experiments by Davis et al. (1947, 1949), histologic lesions were found in 85.5 and 94 percent of the livers examined, in 81 and 89 percent of the ovaries, 80 and 65 percent of the spleens, 77.7 and 73 percent of the kidneys, and 67.7 and 79 percent of the hearts respectively.

The liver and spleen showed pathologic changes of visceral form were arranged by a standard of classification according to their gross alterations proposed by Horiuchi (1961). Table 3 shows the varieties and numbers of affected organs due to grosss alterations; namely diffuse, nodular, granular and mixed variety in liver; and diffuse, nodular and follicular in spleen specimen.

Table 3. Classified Varieties and Numbers of Gross Lesions in Affected Organs of Visceral Lymphomatosis

Liver		Spleen		Ovary		Kidney		Intestinc	
Diffuse: Nodular: Granular: Mixed	<ul> <li>113</li> <li>61</li> <li>34</li> <li>35</li> </ul>	Diffuse: Nodular: Follicular;	46 23 3	Diffuse: Follicular:	11 47	Diffuse: Nodular:	31	Nodular:	15
Total	243		72		58		34		15

Liver: Among 266 chickens showed lesions of visceral lymphomatosis, the livers of 113 (46.5 percent) were diffuse variety of tumors, 61 (25.1 percent) nodular, 34 (14 percent) granular 35(14.4 percent) mixed, and 23 (8.6 percent of total cases) showed grossly mormal.

Diffuse variety: The liver was enlarged uniformly to various degrees, characteristically gray-ish marbleized, mottled appearance, the surface was smooth, and the parenchyma was usually very friable. The affected liveers often retained a reddish-brown color, but of a different shade than normal. For the enlarged livers, the average weight ratio of liver to body weight (L/B) in 38 cases was 14 percent. This was about 5—6 times the normal size.

Nodular variety: In this variety there was less enlargement, the number and size of nodules on liver surface varied greatly; from one to several, and from pin point to egg size. Nodules, unless confluent, were generally spherical except surface flattening and studded in the parenchyma. The nodules were grayish-white, yellowish-gray or reddish-gray in color, depending on the vascularity, and in general it was less vascular than the normal ordan tissue. On cross section the tumors were firm, smooth, and rarely involved necrotic changes. The L/B ratio of this type was 2—6 percent (2—3 times the normal) in 21 cases. If the liver is more than 3 times the normal size it will be possible to palpate the tumorous livers in

any of them before death.

Granular variety: In this type numerous granules of tumor were densely scattered throughout the surface of the liver. They were less than 2 mm. in diameter and rather uniform. The surface of liver was not so irregular but it appeared granular, having many small protuberances. Most granules were uniformly grayish, and composed of small but visible nodules of tumor. Usually, the liver of this variety was a little less friable and smaller than those of the diffuse and mixed varieties. The L/B ratio of this variety was 6—15 percent(4—5 times the normal) in 12 cases.

Mixed variety: This was a combination of two or three types described above. Numerous tumors, irregular in size and shape, were scattered throughout the liver. The surface of the liver was rough and the cut surface showed marbleized appearance too. The L/B ratio of this variety was 8-17 percent in 5 cases.

For the reason that the livers are the most outstanding organs showed gross lymphomatosis lesions, the classification for gross changes was tried with liver specimens by many investigators from earlier days. Burmester(1956) and Jungherr(1952) classified the livers into "diffuse, and "nodular," variety by gross lesions. Feldman(1932) established an another "mixed," variety in addition to above two varieties. Horiuchi(1961) has established a standard of classification for gross and histo-pathological changes occurring in the liver and spleen following the enlargement of liver; presence or absence of distinct nodule formation; and the size of tumorous nodules (granular or nodular) for the reason of unfittable classification by above workers.

Spleen: The enlargement of the spleen was not so severe as that of liver. An average spleen weight to body weight(S/B) ratio of 35 cases was 0.6 percent(2.5 times the normal). About one-half of the specimen ranged 2 times the normal in weight. It seemed a little relationship existed between the enlargement of the liver and that of the spleen. In most cases, the surface of spleen was smooth and some granular protuberances were located at the site of the lymphatic follicles. The affected spleens were usually of a grayish-brown color associated with milky thickening in the capsule. Cross section exhibited minute grayish areas which correspond to hyperplastic lymphocytic aggregates. The gross changes were classified into three types by Horiuchi's classification standard: difluse, modular and follicular. Among 266 cases of visceral

lymphomatosis, the spleen were diffuse in 46(64 percent), nodular in 23(32 percent), follicular in 3(4 percent) of 72 total cases.

Ovary: The affected producing ovaries were easier to detect because the organs were usually enlarged, and the small follicles were obscured by the tumor tissue. But the affected immature ovaries showed diffuse hyperplasis. In the former variety, follicular, in 47(81 percent). In the latter variety, diffuse, in 11(19 percent) of total 58 specimens.

Kidney: The most kidney affected were primarily involved by diffuse grayish enlargement of some of the three major lobes. The diffused were in 31(91 percent) of total 34 specimens. Some showed nodular tumor formation, in 3(9 percent).

Intestine: All specimens had the nodular type of involvement with varied size of multiple tumors.

In other organs such as the lungs, heart, proventriculus, testes, mesentery and peritoneum, even skin were not involved the detectable gross lesion in this study.

### Histopathological Findings of Affected Organs:

The tissues examined under the microscope were 43 cases of visceral lymphomatosis composed of 24 livers, 10 spleens, 3 kidneys, 3 intestines and 2 ovaries.

The tumor cells were lymphoid cells and they varied in size, shape and stainability. Mitotic figures were usually present. The tumors were usually composed of a mixture of cells of various components. The proportions of component cells also were various in all cases. Beside the various proportions of the component cells there were variations in the distribution of the tumor cells. The types of distribution were classified as nodular, infiltrative and diffuse proliferation according to the standard porposed by Horiuchi(1961).

Liver: There were 3 types of visceral lymphomatosis in the lesions. In many cases, more than one type of proliferations were shown, one type usually being predominant. Among 24 cases the infiltrative proliferation was predominant in 12 cases, nodular proliferation in 7 cases, and diffuse proliferation in 5 cases respectively.

In the type of nodular proliferation, the tumor foci were demarcated by fibroid cells. Each nodular focus was round in shape and about 1 mm in diameter. It was suggested that the macroscopic tumors, especially nodular and granular varieties, were clusters of such small foci. The hepatic

cells between the proliferated tumor foci were atrophic. Small numbers of heterophils were usually recognized around the tumor foci and in the inter-lobular connective tissues.

In the type of infiltrative proliferation, the tumor foci were nodular appearance under the lower magnification and about the same size of the foci of nodular proliferation. But the peripheral tumor cells of the nodular foci in filtrated to the adjacent parenchyma. The hepatic cells around thetumor foci were alrophic and there were usually some of heterophils.

In the type of diffuse proliferation, the tumor cells proliferated diffusely in both parenchyma and interlobular tissue, There was no nodular accumulation of tumor foci. It was considered that such distribution of the tumor cells made the appearance of diffuse variety of the tumor macroscopically. The number of heterophils was rather numberous around the proliferated areas.

Spleen: There were 3 types of visceral lymphomatosis in the lesions. Among 10 cases, nodular proliferation was predominant in 5 cases, and infiltrative proliferation in 2 cases respectively. Three cases of the diffuse proliferation were not combined with the other types.

In the type of nodular proliferation, the tumor foci were demarcated dy fibroid cells. Each nobular focus was round in shape and about 1 mm in diameter. The tumor foci were adjacent to or surrounded the central arteries. It was indicated by Horiuchi that the nodular proliferation may originate in white pulps. The red pulps between the tumor foci were compressed and the pulp cords were aggregated.

In the type of infiltrative proliferation, the tumor foci were round in shape and about 1 mm in diameter. They were also found the central arteries. Some tumor foci were fused together and formed a larger and irregular tumor nodule. Under the higher magnification the periphery of the tumor foci was not demarcated by fibroid cells and the tumor cells were infiltrated to surrounding splenic tissues.

In the type of diffuse proliferation, the tumor cells proliferated diffusely in both white and red pulps. The tumor cells were intermingled with pulpular tissues in some areas of red pulps. It was considered that this type of proliferation was resulted in consequence of massive and wide proliferation of the tumor cells originated in white pulps(Horiuchi.)

**Kidney:** Among 3 cases examined, diffuse proliferation was found in 2 cases and nodular proliferation was found in 1 case.

In the type of diffuse proliferation, the tumor cells were diffusely proliferated in inter stitial tissue. It caused pressure to the parenchymal tissue, and then the large amount of parenchymal tissue were destroyed. The remaining tubules were undergone cloudy swelling and vacuolization. Some remaining glomeruli were atrophied and the Bowman's space was dilated. Those manifestations suggested that the parenchymal tissue was disappeared gradually. There was no evidence that the tumor cells invaded directly through the basement membrane of tubules and the Bowman's capsule.

In the type of nodular proliferation, the tumor foci proliferated in the intestitial tissue were demarcated by rather thick layers of fibroid cells. Each nodular focus was round in shape and less than 1mm in diameter. In some areas the kidney showed interstitial fibrosis. The parenchymal tissue affected were less pronounced than the diffuse type.

Intestine: Infiltrative proliferation was found in all of the 3 cases. Each tumor focus was fused together and formed a large tumor nodule. The tumor cells were proliferated in lamina propria, submucosa and the muscle layers. It was suggested that on account of the proliferation of tumor cells in lamina propria, the glands were depressed and disappeared gradually. There was no indication that the tumor cells invaded directly through the basement membrane of the glands. In some areas the mucous membrane was eroded or ulcerated. In the muscle layers the tumor cells infiltrated into the interspace of muscle fibers and formed nodular foci.

Ovary: The two cases examined showed diffuse proliferation. The tumor cells proliferated diffusely in the stroma. The follicles embedded in the tumor tissue were scarce and the remnants were atrophied. Small numbers of heterophils were scattered in the stroma.

### SUMMARY

1). An nanlysis was made of 3,500 postmortem diagnoses for the three years 1961 through 1963 to determine whether there was any actual incidence of avian visceral lymphomatosis in the field. Chickens autopsied, which showed gross alterations were 7.6 percent or 266 cares. The diminished incidence of the disease in second and

third years seemed due to decreased total numbers of chicken flocks year by year for the reason of difficult feed supply.

- 2). Because chickens autopsied in this study were not clearly known of their breeds and lines, no distinct data on the incidnece in various breeds were made. Some exact breeds were in too small numbers to have any statistical significance. Inconceivably, no other types of avian leukosis than visceral lymphomatosis had been observed in any appreciable number in this analysis.
- 3) Pathologic analysis for affected organs was made grossly and microscopically.

In the gross pictures, liver, spleen, kidney, ovary, and in some cases intestine principally showed lesions, but its manifestation was variable in different organs. In such organs, livers were affected more frequently, and spleens followed next. The organs were classified and arranged according to the gross alterations, and among their distribution one-half of livers were in diffuse variety; one-fourths in nodular; about one-sevenths in mixed; and granular variety followed next. In the spleen samples, two-thirds were in diffuse variety; one-fourths in nodular; and follicular only in three cases. Ovaries almost showed follicular lesions, the diffused were less than one-fifths of total specimens. Kidneys were occurred almost in diffuse variety. And intestine showed only nodular tomors.

Microscopically, 42 cases of visceral lymphomatosis composed of 24 livers, 10 spleens, 3 kidneys, 3 intestines and 2 ovaries were examined. The tumor cells were lymphoid cells showing various component in size, shape and stainability. Mitotic figures were usually present. The proportion of the component cells were various in all cases and there were variations in the distribution of the tumor cells. The types of distribution were classified according to the standard proposed by Horiuchi as nodular, infiltrative and diffuse proliferation. In cases of visceral lymphomatosis of the livers and the spleens the types of infiltrative, nodular and diffuse proliferation could be classified. In the cases of the kidneys the types of diffuse and nodular proliferation were observed. In the cases of the intestines and the ovaries the types of infiltrative and diffuse proliferation were observed respectively.

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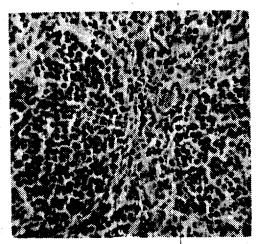


Fig. 1. Nodular proliferation in liver, showing the tumor cells demarcated by fibroid cells. H&E stain, X 430

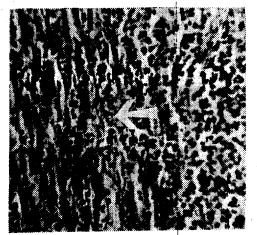


Fig. 2. Infiltrative proliferation in liver, showing obscure border of the tumor foci and the cells infiltrated to the adjacent parenchyma. H&E stain, X 430

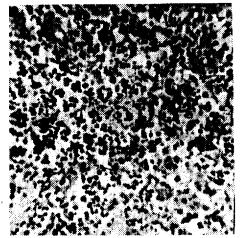


Fig. 3. Diffuse proliferation in liver. The tumor cells proliferated diffusively. H&E stain, X 430

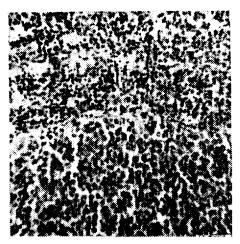


Fig. 4. Nodular proliferation in spleen, showing the large tumor cells clearly demarcated by fibroid cells. H&E stain, X 430

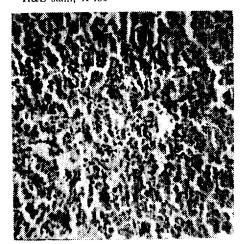


Fig. 5. Diffuse proliferation in spleen. The tumor cells diffusively proliferated in splenic pulp. H&E stain, X4

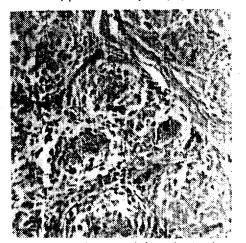


Fig. 6. Nodular proliferation in kidney, showing the tumor cells demarcated by fibroid cells. H&E stain, X 430

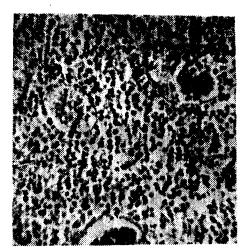


Fig. 7. Diffuse proliferation in kidney. The tumor cells proliferated diffusively in interstitial tissue. H&E stain, X 430



Fig. 8. Infiltrative proliferation in intestine, showing the tumor cells infiltrated to the interspace of smooth muscle fibers. H&E stain, X 430

抄 参

# 職器型淋巴腫症에 闘む 研究

1. 鶏群에서의 淋巴腫症의 發生 및 病理學的所見

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 株
 昌
 身

與自血病群中 特可 臟器型淋巴腫症은 韓國戰爭以後 鷄群의 增加의 미불이 漸次 그 酸生이 많아서 養鶏業界에 相當한 損害量 까치는 것으로 注目되어 왔다. 著者量은 野外鷄群에서의 臟器型淋巴腫症의 發生狀況을 調查하기 ほか여, 1961年~1963年의 3年間에 本大學實驗室과 春川地域의 養鶏揚 및 닭 小賣商에서 刮檢製祭堂 수 있었던 鷄體에 對해서, 다음과 같은 結果를 얻었다.

- 1). 總 3,500의 剖檢例中 266 例(7.6%)에서, 한가지 以上의 職器에서 職器型淋巴腫症의 病變을 觀察하였다.이 比率은 諸外國의 것에 比하여 낮은 率이나, 우리날라의 全飼養鶏群數와 구問의 觀察鷄數量 考慮함배, 本病이 家禽疾病中에서 相當한 比重을 차지할 것으로 豫想된다. 著者들이 檢索한 範圍內에서는 贖器型淋巴腫症 以外의 다른型(自血病群中)은 觀察되지 않았다.
- 2), 肉眼的病變은 肝, 脾, 腎, 卵巢 및 腸에서 觀察되었고, 諸外國의 報告처럼 心, 肺等에서는 觀察되지않았다. 그 發生頻度는 肝이 가장 높았으며, 그 다음으로 脾, 卵巢, 腎, 腸의 順을 따랐고, 各臟器는 單獨 또는 여러가지 組成으로 病變을 나타내었다. 그 肉眼的病變은 Horiuchi(1961)의 分類基準에 따라 各型으로 觀察되었다. 即 肝은 瀰漫型(46.5%), 結節型(25.1%), 顆粒型(14%), 混合型(14.4%)으로, 脾는 瀰漫型(64%), 結節型(32%), 濾胞賦大型(4%)으로, 卵果는 瀰漫型(19%)및 滤胞腫大型(81%), 腎은 瀰漫型(91%) 및 結節型(9%), 腸은 다만 結節型으로各 分類되었다.
- 3). 病理組織學的病變은 Horiuchi(1961)의 分類基準에따라, 24 例의 肝 및 10 例의 脾에있어서, 變稠性增殖型, 結節性增殖型 및 減慢性增殖型으로 觀察되었고, 3 例의 腎에 있어서 瀰漫性增殖型과 結節性增殖型이, 3 例의 腸에있어 시 泛調性增殖型이, 2 例의 卵巢에있어서 瀰漫性增殖型이 觀察되었다. 이와같은 增殖形態을 보이는 木腫纏細胞는 淋巴球系에 屬하는 것으로 생각되는 細胞로, 크기, 모양 및 染色性이 多樣하였다.