LYMPHOMATOSIS, MYELOMATOSIS, AND ENDOTHELIOMA OF CHICKENS CAUSED BY A FILTERABLE AGENT*

II. Morphological Characteristics of the Endotheliomata Caused by This Agent

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Plates 31 to 35

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Begg (1) and Murray and Begg (2), the first to describe endothelioma in chickens, proved that it was caused by a filterable agent. In a previous article (3) we have reported on a transmissible strain of leukosis of fowls (Strain 2) caused by a filterable agent that stimulates to apparently neoplastic growth not only several types of blood cells, but also endothelium.

All filterable agents of chicken sarcoma thus far described are specific in the sense that they produce morphologically characteristic lesions (Claude and Murphy (4)), and the same was found by us to be true for two strains of chicken leukosis. Oberling and Guerin, on the other hand, suppose that the virus observed by them recently (5) was at first the common virus of leukosis of Ellermann and that this virus, while under their observation, mutated into a virus with affinity for mesodermal and ectodermal tissues.

Neoplastic processes are common in chickens and it is erroneous to attribute to the inoculum all types of neoplastic conditions occurring among passages of a virus. Our recent experience with Strain 2 is noteworthy in this connection. While making passages with it two new transmissible sarcomas were isolated. One, a sarcoma composed of spindle and polymorphous cells and abundant collagenous fibers, occurred among the uninjected controls. Its subpassages were free from leukosis. Another, an osteochondrosarcoma, occurred in a chicken injected with Strain 2, and among the birds inoculated with tissues of this tumor there occurred leukosis, osteochondrosarcoma, and leukosis mixed with osteochondrosarcoma.

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Filterable agents of tumors and of leukosis present great variations in the location and morphological appearance of the lesions they produce. For instance, our Strain 2 is capable of producing a variety of anatomically different lesions such as endothelioma, lymphomatosis, myelomatosis, and erythroleukosis. Moreover, each of these lesions occurs in a variety of forms, and the type of disease produced is independent of that of the donor. It is obvious that considerable work is required before the morphological range of a transmissible strain is established; and the occurrence of two instances of non-metastasizing epithelial growth, as observed by Oberling and Guerin among chickens inoculated with their leukosis virus, is insufficient evidence for the assumption that they were caused by the agent of leukosis.

In this article the appearance of endothelial growth as it occurs among passages of Strain 2 will be described, and in the article that follows the results of experimental work aiming to determine the relation of endothelioma to leukosis will be described. Endothelioma, infrequent as a spontaneous disease, appeared repeatedly in a variety of forms. The relationship of these varieties of endothelioma was established by the observation of transitional forms among them, the occurrence of different lesions in the same bird and in different birds that had been injected with material of the same origin.

Nomenclature

Leukosis of fowls may be subdivided into: (1) lymphomatosis, (2) myelomatosis, and (3) erythroleukosis. Lymphomatosis of Ellermann (6) is identical with hemocytoblastic myelosis of Battaglia and Leinati (7). The origin and potentialities of the cells in the disease named by Ellermann lymphoid leukosis and by Battaglia and Leinati hemocytoblastic myelosis still remain to be determined. Battaglia and Leinati consider them mother cells of granulocytes and erythroblasts; that is, hemocytoblasts in the sense of Ferrata (8). The large basophile lymphocytes of Strain 2 seem to be able to produce erythroblasts, myelocytes, and lymphocytes, and therefore function as hemocytoblasts in the sense of Maximow (9). For this reason we have decided to use the term hemocytoblasts as a synonym for the large lymphocytes of Strain 2. Lymphomatosis or lymphoid leukosis of Ellermann includes a group of different diseases in some of which the predominating cells are well differentiated lymphocytes, as in neurolymphomatosis, and in others the predominating cells are cells like large lymphocytes, the potentialities of which are unknown. No attempt will be made to subdivide lymphomatosis until our study of this group of diseases has been completed. Myelocytomatosis is the term used for the disease in which the infiltrations or tumor masses are composed of myelocytes and of almost no other cells. The term endothelioma refers to neoplasms of endothelium including those of the capillaries of the liver, spleen,

and bone marrow. Often it may be impossible to determine whether the tumor cells are of endothelial, mesenchymal, or mesothelial origin. Similar difficulties have been met with in tissue culture studies of these cells, and Fischer states (10)[1] that the endothelial cell of one investigator may correspond to the fibroblast of another investigator.

Material of Study. Causation of Endothelioma. Associated Leukotic Changes

The origin and character of Strain 2 and methods of its transfer have been described (3).

Among the first 150 chickens successfully inoculated with this strain 11 instances of leukosis were associated with endothelioma with giant cells. The 150 instances of leukosis were classified as follows: lymphomatosis (hemocytoblastosis) 72, myelocytomatosis 17, myeloblastomatosis 2, erythroleukosis 8, atypical (mixed) leukosis 38, and untyped 13 (3). 3 instances of endothelial neoplasm unassociated with giant cell formation found in the same series and 17 instances of endothelial neoplasms found among chickens inoculated more recently with Strain 2 are the subject of this study.

Table I shows some significant data on 33 chickens with endothelioma caused by Strain 2, and surveyed in this paper.

The weight of the chickens varied at the time of inoculation from 110 to 1300 gm. All chickens studied were baby chicks or young adult Barred Rock chickens. Four received sublethal doses of x-rays before inoculation, the rest were not irradiated.

All instances of endothelioma observed were associated with either myelocytomatosis or hemocytoblastosis, or both. Since four cases were produced with cell-free agents (dried blood, plasma, plasma filtrate, and frozen blood respectively) and since all but three of the other cases were caused by inoculation of fresh blood, it is evident that endothelioma arises through stimulation of endothelial cells of the host with no implantation of tumor cells. All but four of the chickens whose blood was used for transfers were free from endothelioma, and it is unlikely that even in these four endothelial cells were present in the circulating blood. It is likewise improbable that the large lymphocytes circulating in the blood of chickens inoculated with this

[1] Fischer (10), p. 391.

TABLE I

Data on Chickens with Endothelioma Caused by Strain 2

Chicken		Inoculum			No. of passage	Type of disease in donor	Length of life after inoculation	Blood changes		Type of disease
No.	Weight	Material	Amount	Route				Duration	Morphological	
	gm.		*cc.*				*days*	*days*		
2843	900	Blood	1	iv.	II	Atypical	90	47	*an, mc, h*	*sMc, Enh*
2847	110	"	0.5	"	II	"	88	38	*h, mc, an*	*sMc, Eng*
3146	420	"	0.005	"	II	*lMc*	92	41	*h, an*	*sL, Eng*
3150	490	Plasma	1	"	II	*lMc*	36	18	*er, h*	*aMc, Eng*
3198	—	Blood	1	"	II	Atypical	55	16	*er, h, mc*	Atypical, *En*
2989	800	"	6	"	III	*sL*	67	18	*h, an*	*Eng, sL*
2997	900	"	0.5	"	III	*sL*	64	46	*h*	*sL, En*
3302	1140	"	1	"	III	*sL*	118	7	*an, mc*	*sMc, Eng*
3227	810	"	1	"	IV	*sL*	39	23	*er, h, mc*	*Mc, Eng*
3236	1020	"	0.005	"	IV	*sL*	88	—	Negative	*sMc, Eng*
3241	1140	"	1	"	IV	Atypical	45	30	*an, h, mc*	Atypical, *Mc, Eng*
3113	1200	"	1	"	V	" *Eng*	51	29	*an, h*	" *Eng*
3307	1120	"	1	"	V	"	51	36	*h, an,* later negative	" *Eng*
3349	300	"	1	"	V	"	69	22	*h, an, mc*	" *Eng*
3432	980	"	3	"	V	*sMc*	56	29	*h*	" *En*
3443	1170	Frozen cells	0.005	"	V	*sL*	48	26	*an, h, mc*	" *Enh*
3444	1300	Blood	0.005	"	V	*sL*	63	43	*er, h, mc,* later negative	" *Enh*
3488	200	Blood cells	—	"	V	*sL*	32	—	*an, h, mc*	*sL, Eng*
3489	180	" "	—	"	V	*sL*	63	4	*h, an, mc*	Atypical, *Eng*
3491	210	" "	—	"	V	*sL*	43	23	*an, h*	*sL, En*

3546	200	Dried cells	0.1	iv.	V	*sL*	43	24	*an, h*	Atypical, mainly *Mc, Eng*
3334*	240	Blood	0.2	"	VI	*smc, Eng*	68	18	*an, mc, h*	Atypical, *Enh*
3343	200	"	0.2	"	VI	Atypical, *Eng*	64	—	*an*	*aL, Enh*
3366*	320	"	0.5	"	VI	*lL*	39	13	*er, h, mc*	Atypical, *Enh*
3372*	240	"	0.5	"	VI	*lL*	62	19	*an, h, mc*	*sL, Enh*
3396	230	Plasma filtrate	4.5	"	VI	*lL*	90	43	*an, h*	Atypical, *Ensa*
3406	890	Blood	5	"	VI	Atypical	36	4	*h, an*	*lL, Eng*
3572	750	"	2	"						
		Tumor	—	ith.	VI	"	56	26	*an, h*	Atypical, mainly *L, Eng, Enh*
3338*	200	Blood	0.2	iv.	VII	*sMc, Eng*	36	25	*an, h*	Atypical, *Eng*
3437	840	Tumor	—	im., ith.	VII	*sL*	42	14	*h, mc*	*sL, Eng*
3467	1190	Washed blood cells	—	iv.	VII	Atypical	35	15	*er, h*	Atypical, *Enh*
3500	220	Tumor	—	im.	VII	"	62	—	*h, mc*	*lMc, Eng*
3480	1000	Blood	3	iv.	VIII	*lL*	40	25	*an, h*	*sL, Eng*

* These chickens were irradiated before inoculation with sublethal doses of x-rays.

Abbreviations Used in Table I

Type of Leukosis.—*L* = lymphomatosis (hemocytoblastosis), *Mc* = myelocytomatosis, *E* = erythroleukosis, *Eng* = endothelioma with giant cell formation, *Enh* = endothelioma with hematoma, *Ensa* = endothelioma with sarcoma-like growth, *En* = all other forms of endothelioma. Instances of leukosis in which the involvement of two or more systems was conspicuous are given as atypical leukosis.

Degree of Blood Involvement.—(The blood involvement is described by an adjective used with the abbreviation for the type of leukosis.) *l* = leukemic, *s* = subleukemic, *a* = aleukemic.

Morphological Blood Changes.—(The changes are those seen in blood smears.) *an* = anemia, *er* = anemia suggestive of erythroleukosis, *mc* = myelocytes, *h* = hemocytoblasts (large basophile lymphocytes).

Route of Injection.—iv. = intravenous, ip. = intraperitoneal, im. = intramuscular, ith. = intrathymic.

strain would have changed into endothelium. Moreover, morphological appearances indicated that endothelioma developed *in situ* through proliferation of preformed endothelium.

The blood smears of all but one chicken gave evidence of leukotic changes. The changes were first observed from 11 to 59 days after injection and lasted until death in all but two chickens. In these two the immature cells disappeared from the peripheral blood several days before death. In most cases the presence of numerous polychrome erythrocytes and erythroblasts and occasional basophile erythroblasts in the blood indicated anemia, and in a few cases the relatively large number of basophile erythroblasts suggested erythroleukosis. The presence of large numbers of cells like lymphocytes in the bone marrow, presumably mostly primitive erythroblasts, also often suggested erythroleukosis, but stasis of these cells in internal organs, the most characteristic feature of erythroleukosis, was not observed in the chickens in which endothelioma was associated with leukosis. Large basophile lymphocytes (hemocytoblasts), illustrated in our previous communication (3),[2] were seen in the blood of all but three chickens; their number varied from a few to approximately 100,000 per c.mm. Thus there were no blood changes characteristic of endothelioma; the blood smears showed the presence of either secondary anemia, or erythroleukosis; in others, myelocytomatosis, or hemocytoblastosis, or, most commonly, mixed leukosis (Table I).

The birds died from 32 to 118 days after inoculation. Some were emaciated at the time of death; others were well nourished and died suddenly of hemorrhage from an endothelial tumor.

Morphological Characteristics

In many instances the endothelial growth was small and was discovered only on microscopic examination. In the majority of instances, however, stimulation of endothelium resulted in the formation of tumors varying greatly in size, from a few millimeters to 7 cm. One large tumor attached to the liver is shown in Fig. 1.

Endothelial growth varied in color; it was yellowish grey, grey, or red, and it was often spotted by minute, yellowish, necrotic areas. In contrast to endothelioma, myelocytoma nodules were white, and lymphomatous tumors grey. Necrosis and hemorrhage were common in endothelioma.

The small yellowish areas with endothelioma were composed of necrotic tissue and were surrounded by multinuclear giant cells (Fig. 9). These cells often showed in sections cut at 7 μ from twenty to

[2] Furth (3), Figs. 3 to 5.

fifty nuclei, and it is estimated that many contained hundreds of nuclei (Fig. 6). They resembled those described and illustrated by Murray and Begg (2). Tumors with giant cells were found in eleven of the first 150 birds successfully inoculated with Strain 2, and their occurrence called our attention to the ability of Strain 2 to produce endothelioma. Other types of endothelial growth were not considered in our first report.

Since necrosis was often absent in endothelial tumors with giant cells, it is evident that the formation of the giant cells is not secondary to necrosis. A giant cell tumor free from necrosis is shown in Fig. 6 and a similar neoplasm invading the bone marrow is shown in Fig. 8. In Fig. 6 there are numerous transitional forms between mononuclear and multinuclear giant cells. This photomicrograph, taken of a tumor in the lumbar region, shows invasion of nerves. In sections of giant cell tumors with necrosis, stained by Ziehl-Neelsen's method, no microorganisms were seen.

The nuclei of the endothelial tumor cells and notably those of endothelial giant cells are, in contrast to those of most malignant cells, poor in chromatin. They contain a large, sharply outlined, spherical body stained intensely red with azure, that takes the place of the nucleolus but lacks its basophilia. Giant cells are usually considered as evidence of disturbed cell division; the nuclear characteristics of the endothelial giant cells may be regarded as morphological evidence of cell injury.

Endothelioma with giant cell formation was observed in the following locations: liver, lung, mesentery, intestinal tract, thymus, bone marrow, spleen, ovary, pancreas, skin, eye, kidney, and voluntary muscle. Since only a few tumors of each bird were examined microscopically, the distribution and frequency of this type of lesion cannot be accurately given. It seems to be most common in the liver. The following is the brief history of a fowl with endothelioma showing giant cells.

No. 3302.—The bird was inoculated Dec. 17, 1932, intravenously and intramuscularly with lymphomatous tumor tissue of No. 2930. Blood smears taken repeatedly until Mar. 20, 1933, were negative. On Apr. 4 a few polychrome erythrocytes and erythroblasts and myelocytes were seen in the blood smear. It died Apr. 5. The bird was well nourished and well developed. There was no

tumor at the site of inoculation. Attached to the breast bone there were numerous whitish myelocytoma nodules. Endothelioma with numerous multinuclear giant cells had caused consolidation of the greater part of the lung. The liver was studded with minute white nodules of myelocytoma and contained an occasional yellowish grey nodule of endothelioma with multinuclear giant cells (Fig. 9). In the portal area mesenchymal or endothelial growth was associated with myelocyte formation as shown in Fig. 18. The cavity of the bone marrow was narrowed by spongy, in greater part osteoid, tissue, in the meshes of which were numerous multinuclear giant cells (Fig. 8). The marrow of the femur was spotted thickly with large necrotic areas surrounded by tumor tissue with giant cells. The pulp of the spleen was congested and studded with very large mononuclear cells and giant cells with from two to seven nuclei (Fig. 7). Germinal centers were absent; in their place there were foci composed of large round and multinuclear cells like those that occupied the pulp. These abnormal cells seemed to have originated in the endothelium of the spleen. Their invasive property was made manifest by their penetration through and destruction of the muscular wall of large veins. There were many myelocytes originating in the spleen. In the heart muscle there were small foci of extravascular, diffuse infiltrations composed of tumor cells like those seen in the spleen. The tumors attached to the bones were composed largely of myelocytes that were invading the surrounding fatty tissue. Adjacent to these myelocytomas there were areas of necrosis with multinuclear giant cells.

The endothelial character of some of the tumor cells of Strain 2 was clearly shown when multiple endothelioma was associated with hematoma, as illustrated in Fig. 3 (No. 3467).

In one chicken (No. 3443) bleeding from an endothelioma with hematoma, approximately 0.5 cm. across and situated at the upper part of the right liver lobe, was the immediate cause of death. In No. 3467 the hematomata were surrounded by endothelial cells forming a coherent sarcoma-like growth. These cells surrounded cavities or channels and formed irregular or papillomatous prominences projecting into the lumen (Fig. 4). Within the cavities thus formed hemocytoblasts were abundant and mature erythrocytes were present in small numbers, whereas in the adjacent hematoma erythrocytes were abundant and leukocytes were few. The nuclei of most of the endothelial cells were swollen; many of these cells protruded into the channels or cavities formed by the tumor cells and some of them were detached (Fig. 4). Some of these detached large round cells with large pale stained nucleus and dark stained nucleolus resembled closely the endothelial cells; other cells of smaller size with darker stained nucleus with conspicuous nuclear structure resembled hemocytoblasts, and there were transitional forms between these two types of cells.

The following is the brief history of a fowl having endothelioma with multiple hematomata.

No. 3467.—The bird was inoculated intravenously on Mar. 16, 1933, with washed blood cells of No. 3397. On Apr. 11 there were numerous polychrome and basophile erythroblasts in the blood smear, later myelocytes and basophile lymphocytes appeared in the blood and mitotic figures among the erythroblasts were numerous. It was killed for study on Apr. 20. The mesentery was studded with 1 to 4 mm. red tumor nodules (Fig. 3) formed by the endothelial growth just described. Similar tumor nodules were seen in the spleen (Fig. 3) and ovary. At the aperture of the thorax there was a hematoma of approximately 3 cm. across, the origin of which was not determined. In the liver, nerves, adrenal, and the adjacent sympathetic ganglion, there was mild to moderate infiltration by hemocytoblasts, and mitosis of these cells in the infiltrated tissues indicated multiplication *in situ*. The marrow was almost completely replaced by hemocytoblasts and erythroblasts.

Endothelioma with the formation of channels, but with little or no differentiation of endothelial cells into blood cells, is shown in Fig. 11 (No. 3572).

This section was taken from one of several small (3 to 5 mm.) red tumors that were attached to the rib. On inspection with low magnification the tissues composing the greater part of this tumor resemble young, loose, connective tissue. It is evident with higher magnification, however, that the endothelium of the sinusoidal capillaries of this tumor is hypertrophied and hyperplastic, and that many of the elongated cells in the loose tissue surrounding these capillaries are in appearance indistinguishable from the lining cells of the capillaries. This picture recalls experiments made with tissue culture (*cf.* 10) that showed transformation of endothelial cells into cells indistinguishable from fibroblasts, and it illustrates the difficulties of distinguishing young endothelial cells from young fibroblasts.

Similar but more compact sarcoma-like endothelial growth was seen in the bone marrow of this bird (Figs. 12 and 13). There were numerous areas of hematoma in this organ and the greater part of it was occupied by primitive cells like large lymphocytes. It showed little if any sign of endothelial proliferation. Amidst this hyperplastic marrow there were, however, areas with multinucleated endothelial giant cells. Such alterations show that the agent of Strain 2 has an affinity for both endothelium and primitive blood cells, and that stimulation of each type of cell occurs independently.

In the breast muscle there was a tumor approximately 1 cm. across. This tumor was red at the periphery, grey in the center. It was composed of nests of cells like large lymphocytes in endothelium-lined

spaces and separated from each other by dense connective tissue (Fig. 10).

Thus in this chicken two types of endothelial neoplasms were found; one was formed by sarcoma-like cells, the other by multinuclear giant cells.

Endothelial tumor growth of a different pattern, occurring in No. 3607, is shown in Figs. 15 and 16.

In the solid tumor there were numerous narrow clefts, each surrounded by several layers of hyperplastic endothelium and the lining endothelium was part of this growth. The adult connective tissue matrix that separated the foci of endothelial cells was abundant in some parts of the tumor, scarce in other parts. The endothelial tumor shown in these figures resembles the fibro-angio-endothelioma of Ewing (11).[3] Endothelial neoplasms, mostly with hematoma and varying in size from a few millimeters to about 3 cm. in longest diameter, were found in the liver, lung, thymus, kidney, ovary, mesentery, and muscles of this bird.

Some of the hematomata seen in this bird were evidently of old standing, for one in the liver and another in the kidney were surrounded by fibrous capsules of more than 100 μ in thickness, and in the kidney some of the fibroblasts of this thick capsule were growing into the hematoma.

The origin of some of the hematomata in endothelial growth was not shown by the sections made, but it is possible that this might have been shown through serial sections. Minute endothelial lesions detectable only by microscopic examination may obviously give rise to extensive hematoma. Such was the case in No. 3334, in which there was a hematoma 1.5 × 1.5 × 2 cm. at the aperture of the thorax. At the periphery of this hematoma there were incomplete tubules, mainly composed of cuboidal endothelial cells and containing erythrocytes (Fig. 14).

In different organs of the same bird the microscopic appearance of endothelial growth varied greatly; *e.g.*, in this bird (No. 3334) there was also a solid tumor in the liver, about 1 cm. across, that showed, under the microscope, a sarcoma-like growth similar to that illustrated in Fig. 17.

Tubule formation resembling adenoma or adenocarcinoma, as shown in Fig. 5 (No. 3366), was seen in the liver of several chickens.

[3] Ewing (11), Fig. 132.

The endothelial character of the cells forming such tubules is indicated by their origin in endothelium, by the character of the nucleus of the cells, and by the presence of blood cells in the tubules. The continuity of the cuboidal cells forming these tubules with the endothelial cells of the capillaries of the liver is clearly seen at the edge of the neoplasm where part of the capillaries are formed by normal flat endothelial cells and part by cuboidal endothelial cells. Stimulated endothelium of liver capillaries assumed either gland-like growth as shown in Fig. 5, or grew in a densely packed, perhaps syncytial mass of tumor cells as shown in Fig. 17.

Growth of endothelial or mesenchymal cells was often found in the portal area of chickens inoculated with Strain 2. Fig. 18 (No. 3338) shows a dense mass of cells that resemble cytologically the endothelial cells already described. Adjacent to them are myelocytes and some of the myelocytes can be distinguished from the mesenchymal or endothelial cells merely by the presence of eosinophile granules. This suggests that the myelocytes have arisen by the deposition of granules in the non-granular endothelial or mesenchymal cells. Fig. 18 may be interpreted as morphological evidence of the transformation of mesenchyme or endothelium into myelocytes without the intermediary stage of hemocytoblast or myeloblast (Maximow (9)).[4] In another part of the liver of this bird (No. 3388) the endothelial or mesenchymal cells were elongated and one cell was anastomosed with another.

A section taken from the large tumor shown in Fig. 1 showed only sarcoma-like growth (Fig. 2). The endothelial character of this tumor is suggested by the formation of channels. Its causation by Strain 2 is suggested by the results of the transmission experiments because the two chickens injected with an emulsion of this tumor tissue died of lymphomatosis.

Sarcoma-like growth observed in two chickens after intramuscular transmission of Strain 2 will be more fully described in the article that follows. The growth appeared at the site of injection and distant lesions showed only leukotic alterations.

Ciaccio (12) was the first to describe syncytial tumor growth of endothelium in man, to which he gave the name of syncytial endothelioma. The microscopic appearance of this growth in the lymph node of a man bears, as shown by the photographs of Ciaccio, a striking resemblance to some of the growth caused by the agent of Strain 2. Ciaccio, too, was unable to decide whether the cells forming the tumor were endothelial or mesenchymal cells of the lymph node, but rightly considered that the histogenetical, anatomical, and functional relations of these

[4] Maximow (9), p. 372.

two types of cells are so close as to make it probable that the unknown agent that gave rise to the tumor might have stimulated either type of cell.

Diffuse infiltration by detached cells resembling large lymphocytes and by mesenchymal or endothelial cells forming dense, perhaps syncytial masses were not uncommon in the same bird and in the same organ. In some organs, such as nerves, the infiltrating cells were almost invariably detached. Occasionally, however, the cells infiltrating the nerves were large, palely stained, and appeared to be connected. Both types of infiltration occurring in the same nerve are shown in Figs. 21 and 22; the cells at the periphery of the nerve are the anastomosing cells and appear to have orginated either in perineural mesenchyme or in endothelium. The cells that infiltrated the central parts of the nerve resembled large lymphocytes.

A tumor composed of large, round cells (Figs. 19 and 20) (No. 3432) was found adjacent to the thyroid gland. It measured approximately 1 cm. across, contained thymic tissue, and apparently originated in or about a thymic lobe. The large size of the cells forming this tumor, their pale stained nucleus with compact dark stained nucleolus, and the numerous binucleated cells distinguish them from primitive blood cells. There was no evidence of maturation of these cells into blood cells. Similar tumor growth was seen in the mesentery of other chickens invading the proventriculus, gizzard, duodenum, and pancreas.

Evidence for the view that endothelium stimulated by the agent of Strain 2 produces blood cells is furnished in Fig. 23 (No. 3489). Here is seen the development of hemocytoblasts from endothelium within a sinusoid of the bone marrow lined by the activated or neoplastic endothelium. This sinusoid is surrounded by young, granular leukocytes. This picture, as well as Figs. 24 and 18 suggests, furthermore, that myelocytes may arise by the deposition of acidophile granules in endothelial or mesenchymal cells. By contrasting Fig. 23 with the figures shown in the report of Ratcliffe and Furth (13) on the pathogenesis of erythroleukosis, it can be observed that Strain 1 activates the primitive blood cells and not the endothelium of the marrow, whereas Strain 2 may activate both types of cells.

Sections are less suitable than blood smears for the study of the

maturation of hemocytoblasts into various types of blood cells. Blood smears of chickens inoculated with Strain 2 show abundant transitional forms between hemocytoblasts, on the one hand, and typical erythroblasts, myelocytes, and lymphocytes, on the other hand.

DISCUSSION

Type of Cells Stimulated by Strain 2. Hemopoietic Potentialities of Mesenchyme, Endothelium, and Primitive Blood Cells.—Sufficient data are not available to present a clear-cut picture of the pathogenesis of Strain 2, which seems to cover almost the entire field of blood formation. It causes a tumor-like multiplication of mesenchyme or endothelium. These adherent, or perhaps syncytial, cells either remain undifferentiated or they become detached and transformed into cells like large lymphocytes (hemocytoblasts). In blood smears of chickens injected with Strain 2 there are numerous transitional forms between these large lymphocytes, on the one hand, and erythroblasts, myelocytes, and small lymphocytes, on the other hand. Myelocytes appear also to arise in cells of endothelial type without the intermediary stage of large lymphocytes. Most myelocytes develop, however, by mitotic division of myelocytes, and most erythroblasts develop by multiplication of primitive erythroblasts.

The endothelial, mesenchymal, or mesothelial origin of the growth often cannot be determined (*cf.* the studies on the morphological and functional relationship of endothelium to mesenchyme (9, 10, 14)). It is evident, however, that several genetically closely related types of cells may become stimulated by the agent of Strain 2.

Sabin, Doan, and Cunningham bring evidence to show (*cf.* 15) that lymphocytes and granulocytes arise from mesenchyme, erythroblasts from endothelium. Since endothelium of the blood-forming organs, according to the now prevailing view, is flattened mesenchyme, the distinction between the views of these investigators and those who believe that all blood cells are derived from a single type of fixed cell has little significance. Our observations are in accord with those of Sabin, Doan, and Cunningham and indicate that erythroblasts arise intravascularly in the chicken, myelocytes and lymphocytes extravascularly, usually perivascularly. The mother cell of all blood cells is obviously either mesenchyme or its differentiated form, the endo-

thelium of the blood-forming organs, and the type of cell produced depends upon the growth stimulus. Under ordinary conditions the endothelium of the blood-forming organs of adult chickens is not hemopoietic, and blood cells arise mainly by multiplication of primitive blood cells. Nevertheless, when stimulated by agents such as that of Strain 2, the endothelium may become hemopoietic. It is noteworthy that in the chick embryo the formation from endothelium of large round cells like hemocytoblasts may be observed in numerous organs. Nonidez (16) has described it in the ovary, testis, meso- and metanephros, adrenal, and epididymis.

Local conditions influence the type of growth produced by the agent of Strain 2. Certain organs or parts of organs have a predisposition for characteristic growth; *e.g.*, the cells infiltrating the nerves and myocardium are almost exclusively large lymphocytes, the tumors attached to the bones are composed of myelocytes, and of almost no other cells, and the cells formed within the sinusoidal capillaries of the marrow are largely primitive erythroblasts. The tumors of the mesentery are usually composed of very large round cells or giant cells. The growths induced in the breast muscle and thymus by injections of tissues containing the agent of Strain 2 are usually hemocytoblastic, and occasionally hemocytoblastic and sarcoma-like.

In order to obtain conclusive evidence of the origin of growths produced by the filterable agents causing tumors and leukosis as also of the potentialities of endothelium, mesenchyme, and primitive blood cells stimulated by these filterable agents, studies on living tissue (*e.g.* in culture, or in the Sandison-Clark chamber) are desirable.

Histological Types of Endothelioma in Man and in Fowl.—Most histological types of endothelioma described in man have been observed in chickens injected with Strain 2. Ewing's "interfascicular" type has been described here as sarcoma-like, his "alveolar" type as glandular, his "plexiform" and "diffuse" types as sarcoma-like or syncytial. Giant cells are rare in endothelioma of man, common in endothelioma of chickens. Brosch and Ewing describe giant cells of the Langhans type in endothelioma of man (*cf.* Ewing), and some of the multinuclear giant cells of avian endothelioma resemble closely those of an avian tubercle. Endothelioma of the bone (endothelial myeloma of Ewing) is rare in chickens but myeloma (myelocytoma) is common. Evidence has been presented above that the myelocytes of chickens may originate in mesenchymal or endothelial cells under the stimulus of the same filterable agent that causes endothelioma. The

similarity between syncytial endothelioma of man (*cf.* Ciaccio) and of chickens has already been mentioned. The association of endothelioma with leukemia is rare in man. Ewing observed a case of lymphatic leukemia associated with alveolar endothelioma. The tumors arising in endothelium of lymph nodes and usually classified as large-cell lymphosarcoma may be considered as a link between leukemia and endothelioma.

CONCLUSIONS

When stimulated by a filterable agent of leukosis of chickens (Strain 2), endothelium may undergo seemingly unrestricted growth. These neoplasms of endothelium are usually unaccompanied by the formation of blood cells. Occasionally they produce hemocytoblasts, discharged like those of the normal marrow into vascular channels as also myelocytes about the vessels.

The same agent that stimulates endothelium also stimulates erythroblasts, myelocytes, and hemocytoblasts to unrestricted growth without obviously affecting the endothelium; and the association of endothelioma and leukosis is the result of stimulation of several types of cells by a single virus. Myelocytes appear also to develop from mesenchymal or endothelial cells without the intermediary stage of hemocytoblasts.

It is often impossible to determine whether the neoplasms caused by the virus of Strain 2 are of endothelial or mesenchymal origin, and it is possible that both types of cells may be stimulated by the same virus. Types of sarcoma like those described by Rous are not produced by the virus of Strain 2.

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EXPLANATION OF PLATES

All sections were prepared from tissues fixed in Zenker-formol solution and were stained either with hematoxylin-eosin or with hematoxylin-eosin-azure solutions. The magnifications given are approximate.

PLATE 31

FIG. 1. (No. 3396.) A large tumor (*a*) attached to the left lobe of the liver, a small tumor (*b*) in the upper part of this lobe, and two tumors (*c*) at the site of the ovary.

FIG. 2. (No. 3396.) Microscopic appearance of the endothelial tumor of the liver shown in Fig. 1. The tumor cells form rows and tubules. × 100.

FIG. 3. (No. 3467.) Multiple endotheliomata with hematomata of the liver.

FIG. 4. (No. 3467.) Microscopic appearance of the small irregular cavities in the endothelial growth shown in Fig. 3. The cavities are lined by endothelial cells and several of these cells are detached. The cavities are filled chiefly with hemocytoblasts. × 300.

PLATE 32

FIG. 5. (No. 3366.) Endothelioma of the liver composed of cuboidal cells that are continuous with the endothelium of the liver capillaries. × 300.

FIG. 6. (No. 3546.) Endothelioma with giant cell formation infiltrating nerves. × 100.

FIG. 7. (No. 3302.) Diffuse endothelioma with giant cell formation in the spleen. × 300.

FIG. 8. (No. 3302.) Endothelioma with giant cell formation in the marrow cavity of the femur. × 150.

FIG. 9. (No. 3302.) Endothelioma with giant cell formation in the liver. × 50.

PLATE 33

FIG. 10. (No. 3572.) Microscopic appearance of a red tumor occurring in the muscle. Abundant connective tissue separates nests of intravascularly located hemocytoblasts. × 150.

FIG. 11. (No. 3572.) Microscopic appearance of a small endothelial tumor that was attached to the rib. The endothelium of the sinusoidal vessels is greatly hypertrophied and the elongated cells in the loose tissue surrounding the sinusoidal vessels are in appearance indistinguishable from the endothelial cells that line these channels. × 300.

FIG. 12. (No. 3572.) Shows two types of lesions in the bone marrow: (*a*) sarcoma-like growth of endothelial cells; (*b*) hyperplasia with formation of erythroblasts and cells like large lymphocytes. × 80.

FIG. 13. High magnification of the sarcoma-like growth of endothelial cells shown in Fig. 12. × 250.

PLATE 34

FIG. 14. (No. 3334.) Endothelial growth at the edge of a hematoma. × 250.

FIGS. 15 and 16. (No. 3607.) Endothelial growth with the formation of narrow clefts surrounded by tumor cells. Abundant connective tissue separates the foci of endothelial growth. Magnifications: Fig. 15, × 100; Fig. 16, × 300.

FIG. 17. Extensive endothelial growth in the liver of No. 3490. × 300.

FIG. 18. Mesenchymal or endothelial growth in the portal area of the liver (No. 3338). There are numerous eosinophile granules in many of the tumor cells. × 300.

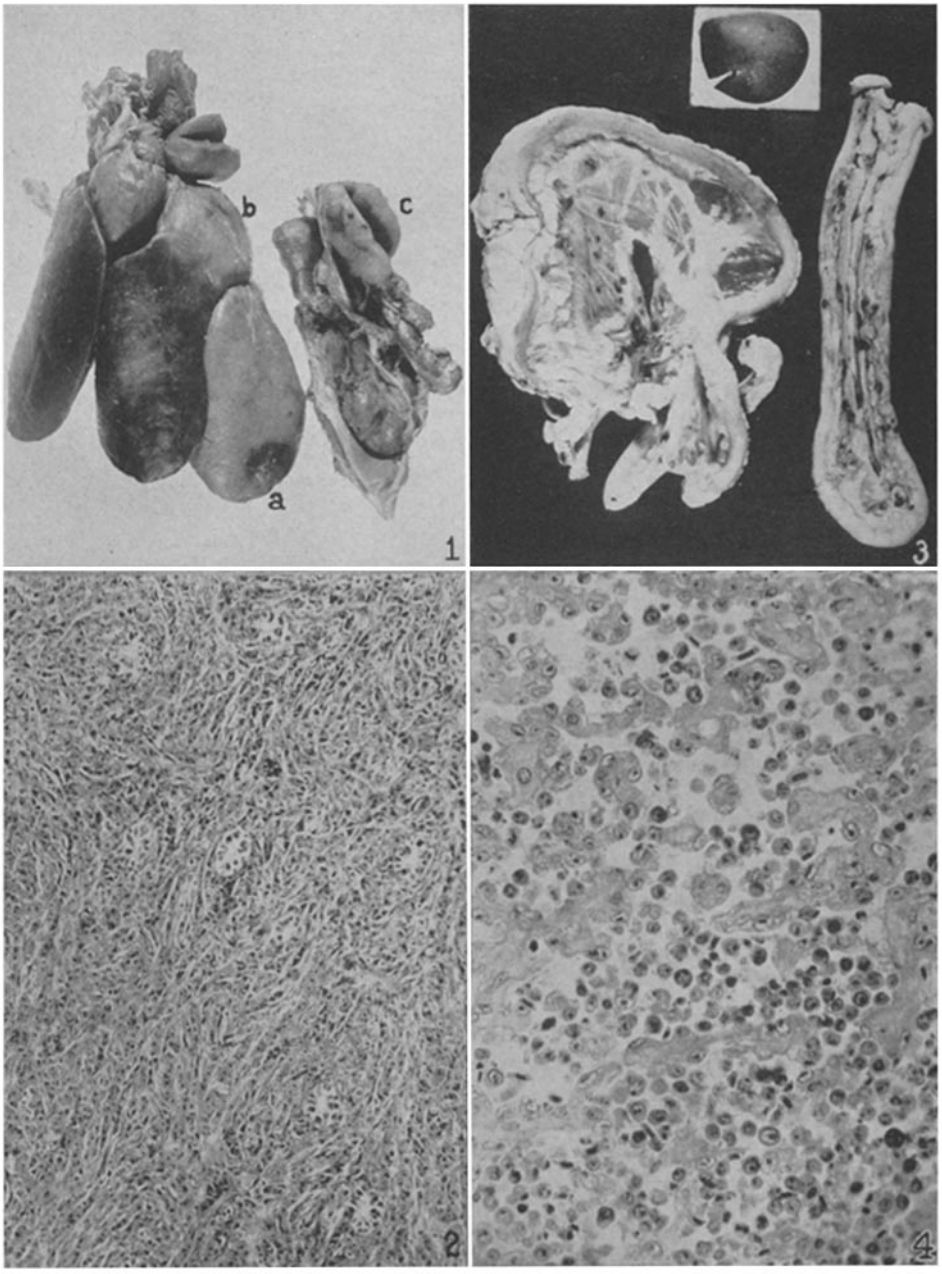
PLATE 35

FIGS. 19 and 20. (No. 3432.) Large round cell sarcoma of the thymus, probably of endothelial origin. Magnifications: Fig. 19, × 150; Fig. 20, × 700.

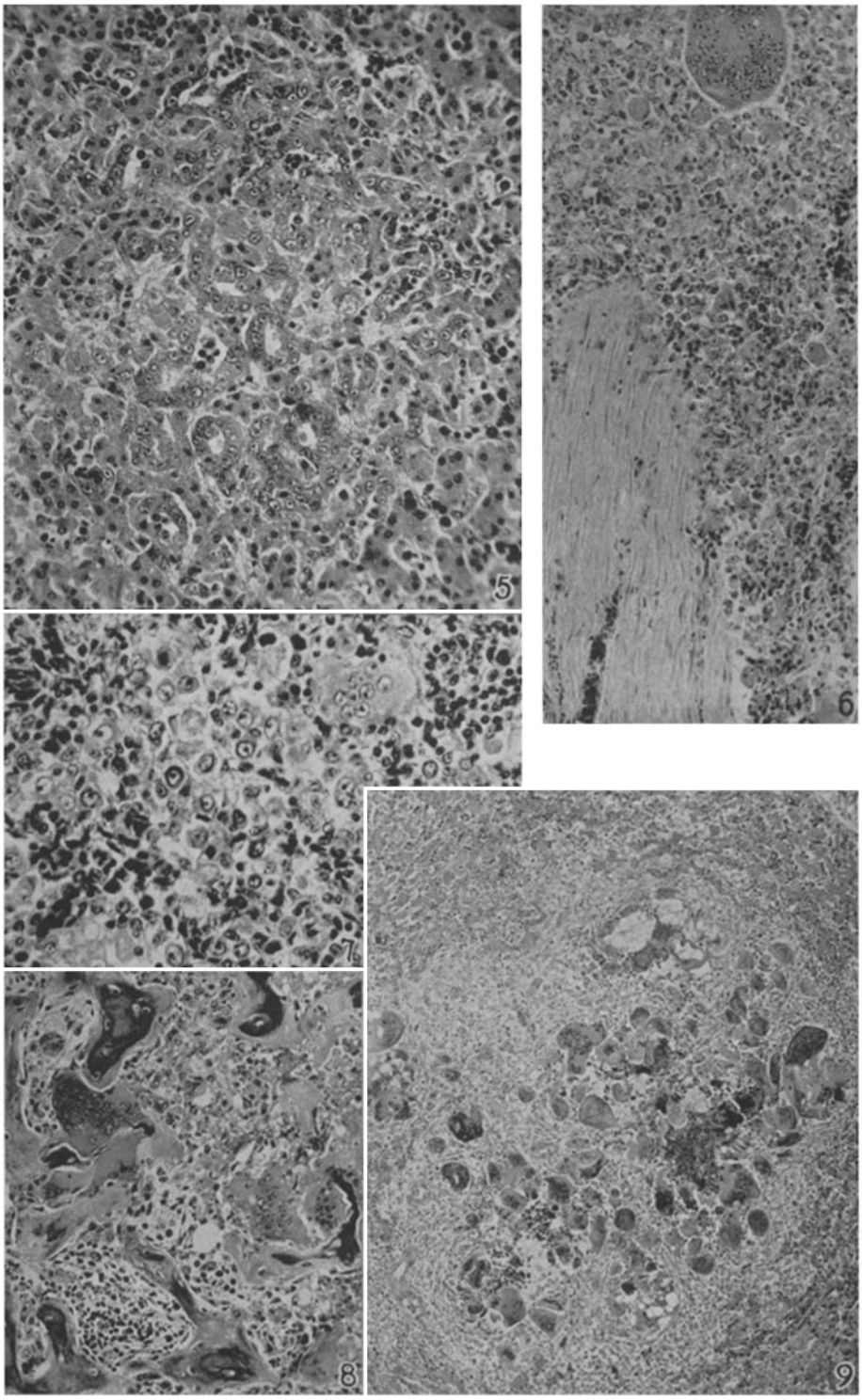
FIGS. 21 and 22. (No. 3571.) Two types of infiltration in nerves, hemocytoblastic and endothelial or mesenchymal. In Fig. 22 hemocytoblastic infiltration in the lower part of the field, endothelial or mesenchymal in the upper part. Magnifications: Fig. 21, × 100; Fig. 22, × 200.

FIG. 23. (No. 3489.) Hypertrophy and hyperplasia of the endothelium of a sinusoidal capillary of the bone marrow. Detached endothelial cells and hemocytoblasts occupy the sinusoid that is surrounded by myelocytes. × 400.

FIG. 24. (No. 3489.) An area of bone marrow with formation of myelocytes from endothelial or mesenchymal cells. × 350.



(Furth: Virus causing lymphomatosis of chickens. II)



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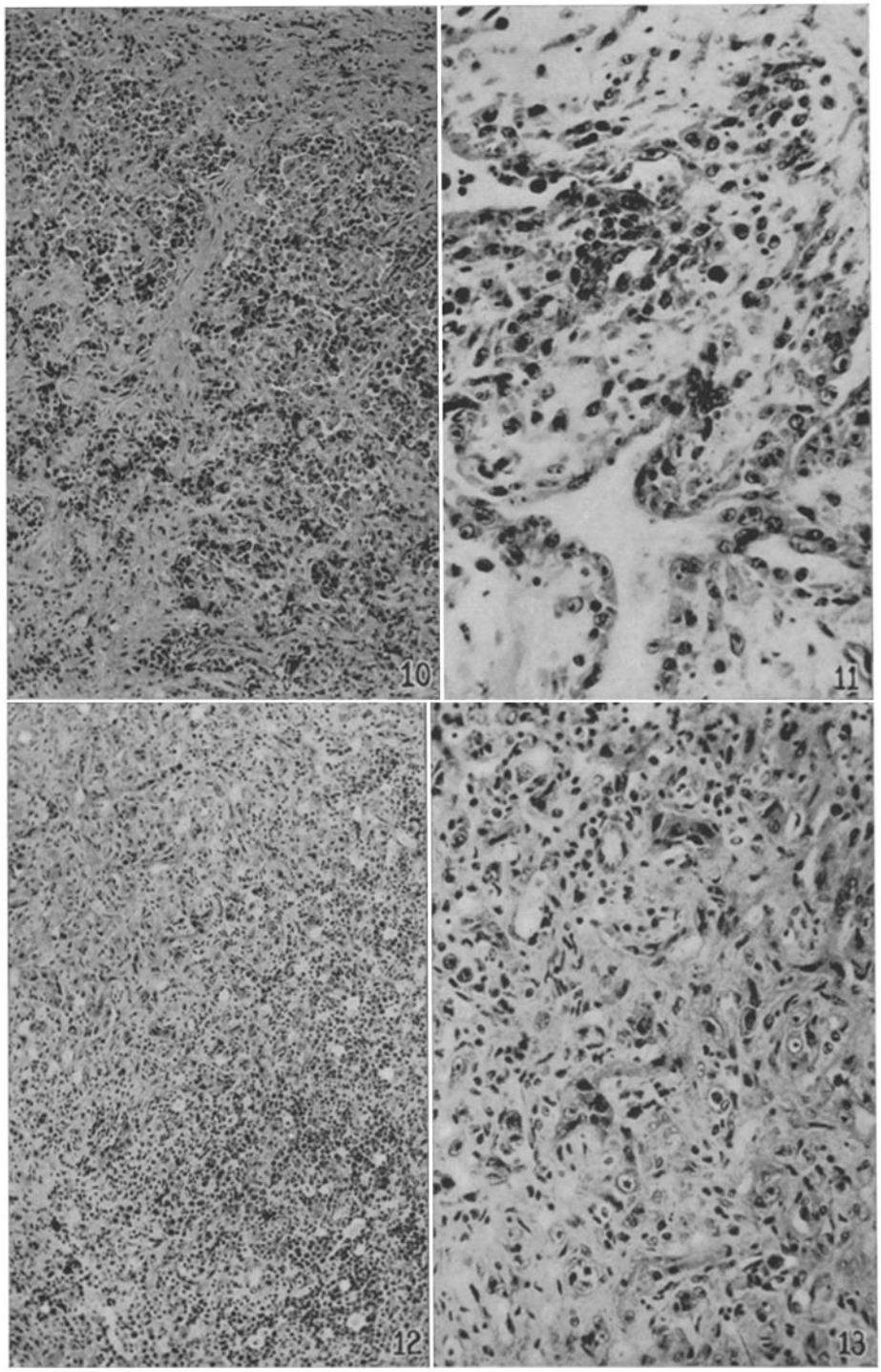

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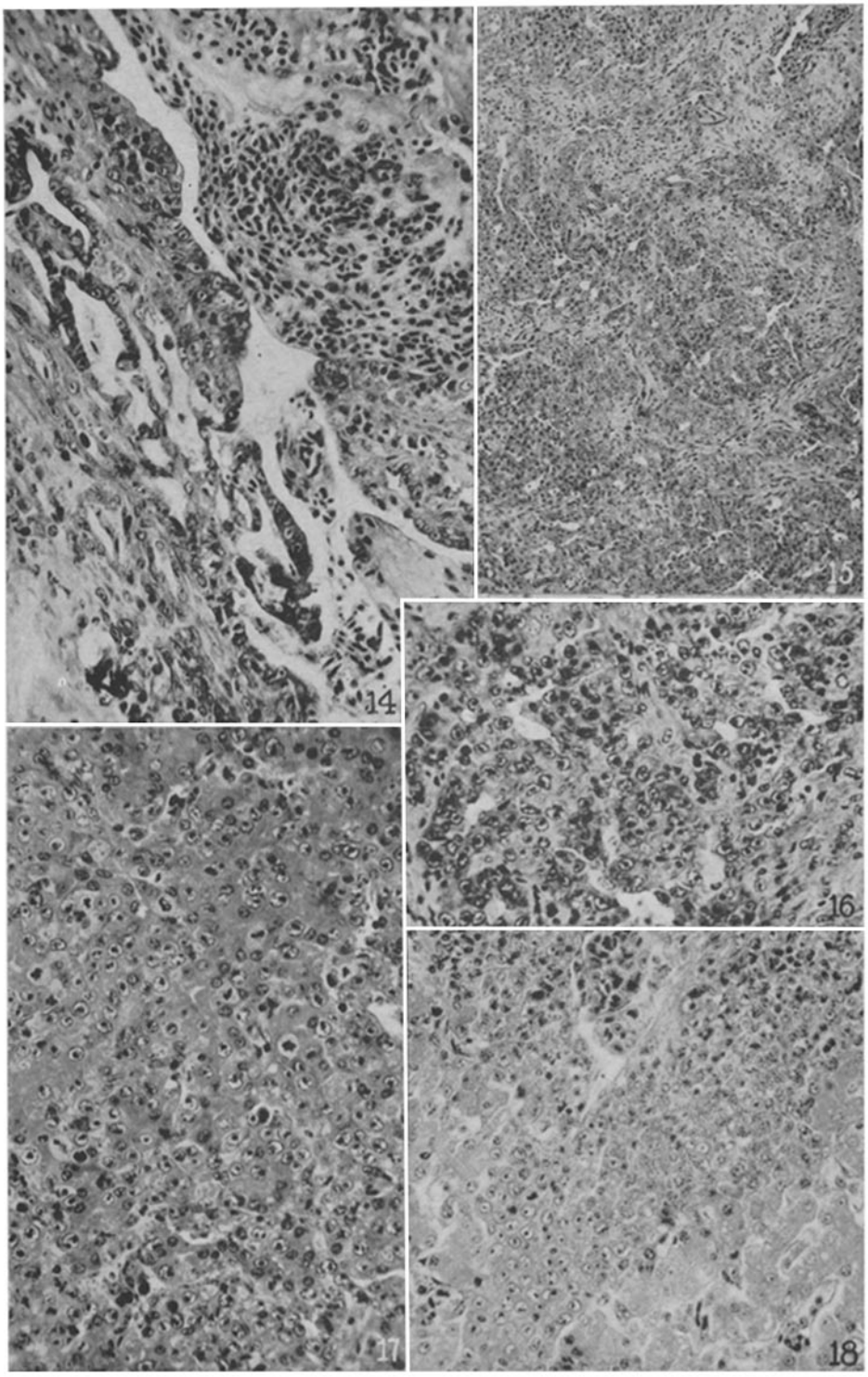
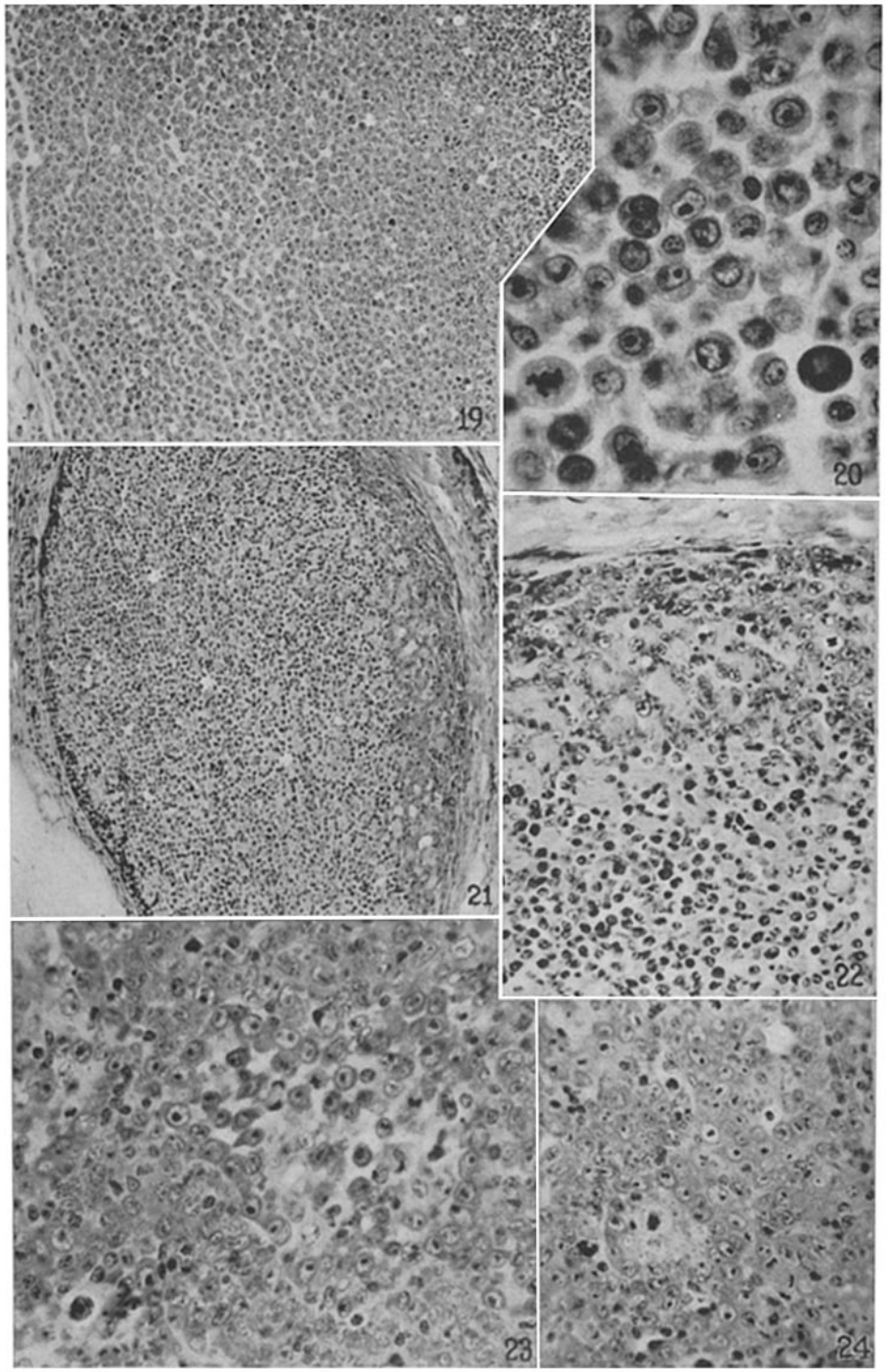


(Furth: Virus causing lymphomatosis of chickens. II)



(Furth: Virus causing lymphomatosis of chickens. II)