Transplantable Lymphosarcoma of the Chicken

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EDITORIAL NOTE: On account of conditions caused by the war it has not been possible to exchange communications with Professor Pentimalli without long delays. Matters concerning his manuscript could not be taken up through the usual means of correspondence. In preference to an indefinite postponement of the publication of his observations the Committee has taken the liberty of issuing an abstract of his paper.

ABSTRACT

The tumor described in this paper arose in a Leghorn chicken selected for examination because of its pale comb and emaciation.

Examination of the blood revealed a severe change of erythroblastic type with the presence of numerous pathologica elements difficult of classification. In nuclear structure the cells resembled at times lymphoblasts and at other times, hemocytoblasts. Nucleoli were present and the cytoplasm was strongly basophilic. Many of the cells were in mitosis. No granulocytes were observed.

At autopsy the liver was found to be enlarged, weighing 130 gm. It was covered with grayish white spots, measuring 0.1 to 2.5 cm. in diameter, separated by hepatic tissue of normal color. On section the organ appeared to be transformed into a mass of grayish white nodules of various sizes. On microscopic examination the normal hepatic tissue was found to be reduced to narrow bands separating the neoplastic nodules. The latter consisted of a loose connective tissue stroma containing accumulations of large round tumor cells with a nucleus rich in chromatin and showing nucleoli. The cytoplasm of some cells was scanty and of others, abundant. In some cells the chromatin was less profuse and was distributed in the form of a network, while the cytoplasm stained less deeply. Mitosis was observed in cells of both types. The arteries of the liver were intact, but the veins frequently showed destruction of their walls, and neoplastic tissue was present in the lumen at some points. In some places the small periportal veins were obliterated by compression. Although there were few blood vessels in the neoplasm itself necrosis was not observed.

The spleen measured 3.0 by 2.5 cm. in its greatest diameters. It had a uniform reddish brown color and did not contain nodules such as those present in the liver. The ovaries contained small whitish nodules. The femoral bone marrow was deep red in color and was removed with difficulty. There were no changes of significance in the lungs or other organs.

Smear preparations of the tumor tissue, stained by the May-Grünwald and Giemsa technic showed the 2 cell types even more clearly than microscopic sections.

Most of the cells were of variable size, containing a nucleus rich in homogenously distributed chromatin and 1 or 2 nucleoli. The cytoplasm, which was scanty or abundant, was intensely basophilic and free from granules. A halo of lighter cytoplasm was sometimes seen around the nucleus. Mitoses were numerous. These cells, with definitely pathological features, resembled lymphoblasts, but differed from them by their larger size.

The second less numerous type of cell was much larger, containing a coarse chromatin network, with clear spaces between the fibrils. The cytoplasm was much more abundant than that of the previously described first type of cells. It was slightly basophilic and contained no granules. Mitosis was frequent. These cells appeared to correspond to large atypical monoblasts.

The difference between these 2 types of cells lay in the formation of the nuclear chromatin. After transplants to the pectoral muscles of chickens the 2nd cell type, following intravenous injection of lithium carmine, was found to contain a single granule of the dye, while the cells of the first type showed no such inclusions.

The microscopic appearance of the kidneys was identical with those seen in the liver. The parenchyma was destroyed by compression and the tumor tissue had infiltrated between the renal tubules producing a picture characteristic of leukemic invasion. The small nodules in the ovary were composed of cells of the same type.

The normal structure of the spleen was so greatly altered that not even remnants of the follicles could be distinguished. The venous sinuses were barely visible. Erythroblastic cells of various degrees of maturity filled the sinuses. Hemocytoblasts, a few myelocytes, Rieder cells, and normal erythrocytes were also present. The bone marrow was transformed into a firm tissue rich in immature erythropoietic and myelogenic cells. This tissue contained no evidence of blood vessels or sinuses. Some cell groups had characteristics similar
to those of the neoplastic tissue found in the liver, but no nodular formations were seen in the bone marrow.

Transplantation experiments.—Four chickens were inoculated in the pectoral muscle with an emulsion of hepatic tissue from the chicken bearing the original tumor. Five others were inoculated in the vein of the wing. Six chickens were inoculated intravenously with a protein preparation made according to a method previously described by the author. To prepare this material an extract of the liver was precipitated with 0.3 and 0.6 saturated solutions of ammonium sulfate, the precipitate was washed with ammonium sulfate, dissolved in a phosphate buffer solution at pH 7.4, precipitated, redissolved, and dialyzed.

No tumors developed in any of the chickens which had been injected intravenously with either the emulsion of the tumor or the solution of the precipitate from the extract. In the group of 4 chickens inoculated in the pectoral muscle with emulsion 1 tumor arose. This appeared 8 days after inoculation in the muscle. It measured 2 by 3 cm. and was soft in consistency. Histologically the cellular structure was identical with that of the original neoplasm. The muscular tissue was invaded by infiltration between the fibers. The 2 types of cells were present. Blood vessels were few, consisting of thin walls sometimes composed of a single layer of endothelial cells.

Four chickens were inoculated in the pectoral muscle with an emulsion of this transplanted tumor. Tumors developed at the site of inoculation and the neoplasm was carried through 2–3 transplant generations in a year. In each of these the characteristics of the primary lesion were reproduced. The tumor reached the size of a walnut in 1 or 2 weeks. It then infiltrated the entire pectoral muscle and caused the death of the fowl in 4 to 8 weeks.

The tumor rapidly acquired such virulence that by the 8th transplant generation practically 100 per cent takes were obtained. After the 8th transplant generation there was an apparent reduction of virulence, but this was largely regained later. A certain number of chickens, however, were resistant to the transplanted tumor.

The internal organs of animals bearing transplanted tumors were usually normal except for occasional slight enlargement of the spleen, which, however, was normal in appearance and consistency. Metastatic growths were seldom observed. Among 92 animals which developed tumors secondary deposits were found three times in the liver and kidneys, twice in the lungs and heart, and once in the spleen. Histological examinations of the organs probably would have shown more widespread and more numerous metastases.

In addition to the 5 chickens inoculated intravenously with an emulsion of the original tumor, 2 chickens were injected intravenously with an emulsion of the 4th transplant generation of the tumor, 6 with an emulsion of the 9th generation, and 4 with an emulsion of the 13th generation. None of these developed tumor in any organ.

The blood of animals receiving inoculations in the pectoral muscles, as well as of those injected intravenously, was examined at intervals of 6 or 7 days. Only transient changes were observed. These consisted of monocytosis without immature cells, and of anemia.

Filtration experiments.—The filterability of the tumor was tested at the 3rd, 8th, and 12th transplant generations. The results with Berkefeld and filters were negative.

Desiccation experiments.—Attempts were made to transmit the tumor with desiccated material from the 4th, 8th, 12th, 18th, and 20th transplant generations. All of these experiments gave negative results.

Resistance of the tumor to glycerine.—This was tested at the 20th generation by keeping the neoplastic tissue in glycerin for 8 days. The results were negative.

Discussion and Conclusion.—Professor Pentimalli reviews a number of papers by investigators who have studied transplatable tumors of the chicken and the leukoses of the fowl. The papers referred to are cited in the references. The editors have added several references to this list.

After discussing the characteristics of previously described tumors Professor Pentimalli concludes that the neoplasm described in this paper is the first example of transplatable lymphosarcoma of the chicken, and that it does not contain a filterable agent.

REFERENCES
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