

AN OUTBREAK OF UNKNOWN ETIOLOGY AMONG CHICKENS PRODUCING LEUKEMIA AND DIFFERENT KINDS OF TUMORS *

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During the winter of 1962, an outbreak characterized by high morbidity and mortality, loss of weight and egg production was recorded among 2,000 white Leghorn chickens, housed in Warrenton, Virginia. Most of the chickens were between eight and 12 months old, although some were 18 months old.

Most of the sick or dead chickens had subcutaneous or abdominal tumors firmly attached to the organs, anemia, pathologic changes of the red cells, mild erythroblastosis, myeloblastosis or high lymphocyte counts in peripheral blood. No osteopetrosis, nerve involvement or ocular necrosis was observed. The disease was initially diagnosed as a highly virulent form of fowl leukosis as had been described in the literature.¹⁻²

Material and Method

Our methods included pathological investigation, chorioallantoic (CAM) inoculation, inoculation of the tissue culture using chicken embryo fibroblast, neutralization tests, and inoculation of experimental animals.

Pathological findings: Pathologically, the tumors were extremely varied. For instance, the solid tumor on the neck of chicken no. 5 (Figure 1) consisted of large round cells and was identified as a squamous carcinoma (Figures 2 and 3). Chicken no. 15 had a solid mesenteric tumor, reddish in color, similar to a leiomyoma (Figures 4, 5, and 6). Another chicken had multiple tumors of the mesenter, ovaries and kidneys which were diagnosed as myxosarcomas (Figures 7, 8, 9, and 10). A blood smear from chicken no. 3 showed typical myeloblastosis (Figure 11). Chicken no. 2 had lymphocytomas of the liver and kidney, and its blood did not clot (Figure 12).

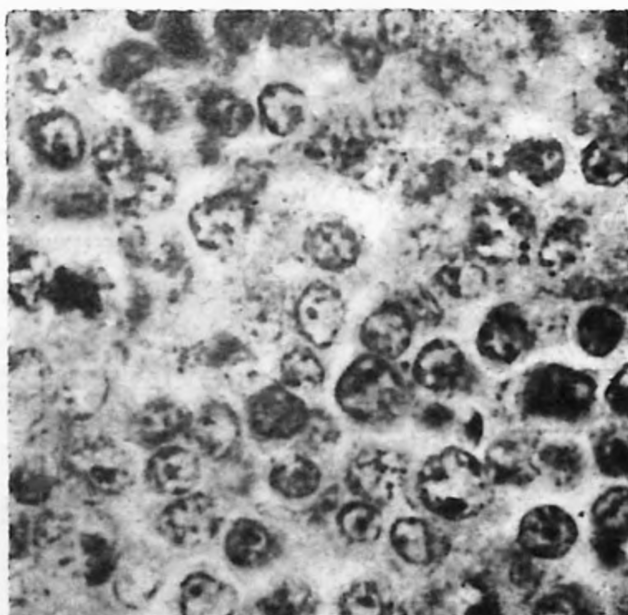
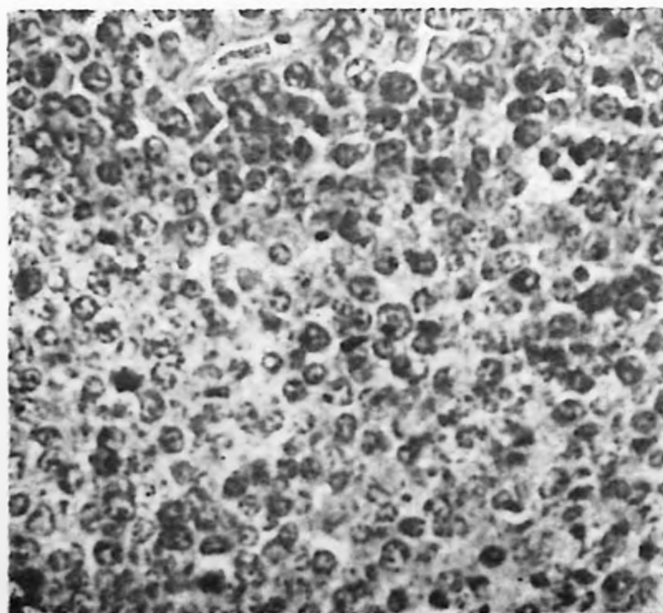
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Chorioallantoic membrane (CAM) inoculation: All experiments in this series were done with cell-free materials. Twelve-day-old embryonated chicken eggs were inoculated on CAM with different tumor materials and small or large tumors were produced which did not resemble the poxes produced by RSV-Bryan strains. Five days was the optimal time for development of the lesions



Figure 1. Solid neck tumor chicken no. 5.



Figures 2 and 3. (x250 and x400 respectively). Histopathology of the tumor showing rounded cells of «squamous carcinoma», chicken no. 5.



Figure 4. Single mesenteric solid tumor, chicken no. 15.

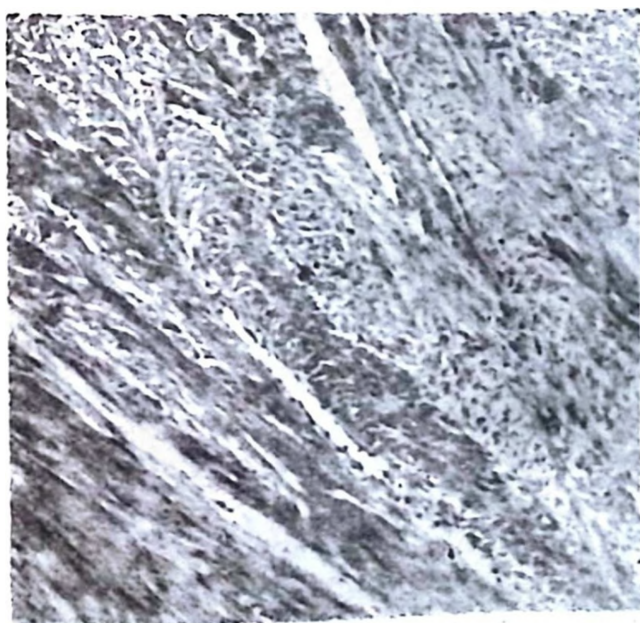
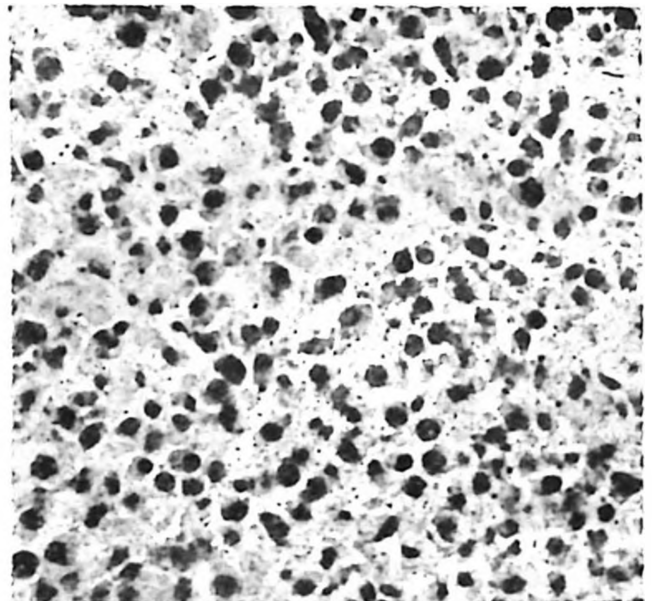
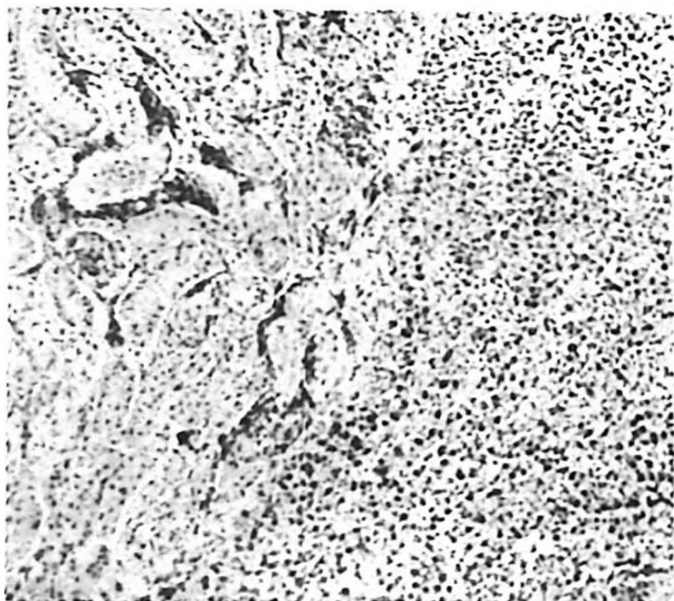


Figure 5 and 6. (x250 and x400). Mesenteric solid tumor showing leiomyoma-like cells, chicken no. 15.



Figure 7. Multiple tumors throughout the viscera, chicken no. 1.



Figures 8 and 9. (x90 and x400, respectively). Kidney myxosarcoma, chicken no. 1.

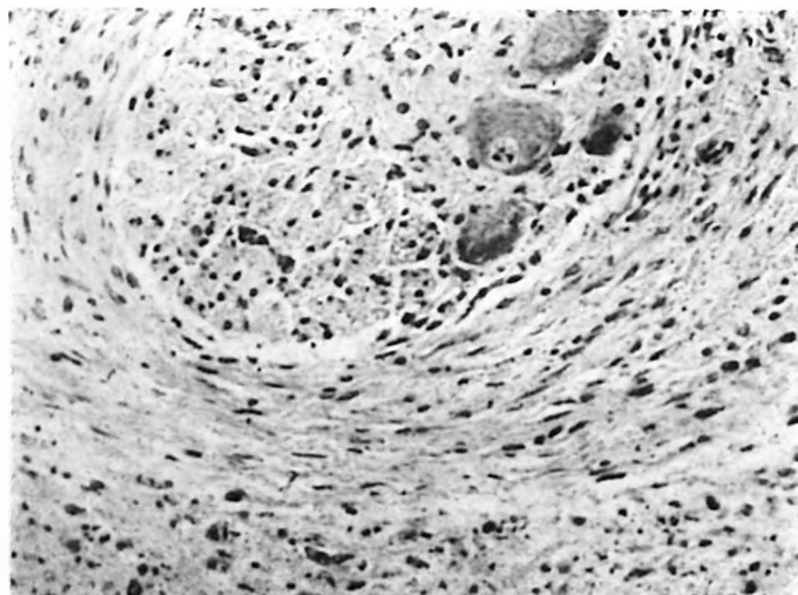


Figure 10. x250. Mesenteric tumor, myxosarcoma, chicken no. 1.

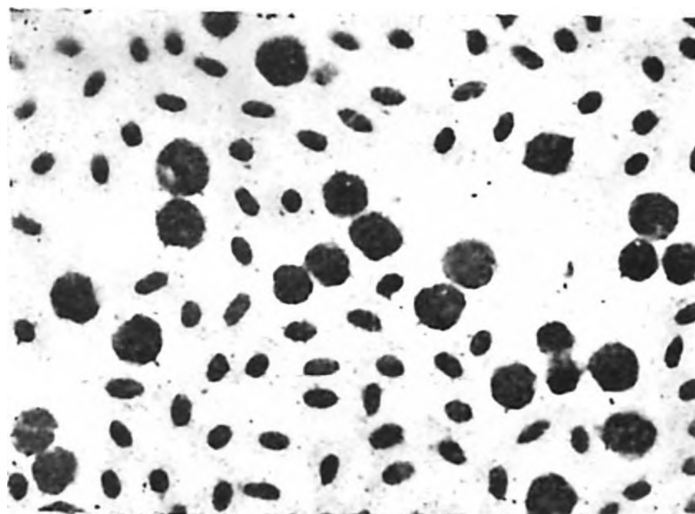


Figure 11. x640. Blood smear showing myeloblastosis, chicken no. 3.

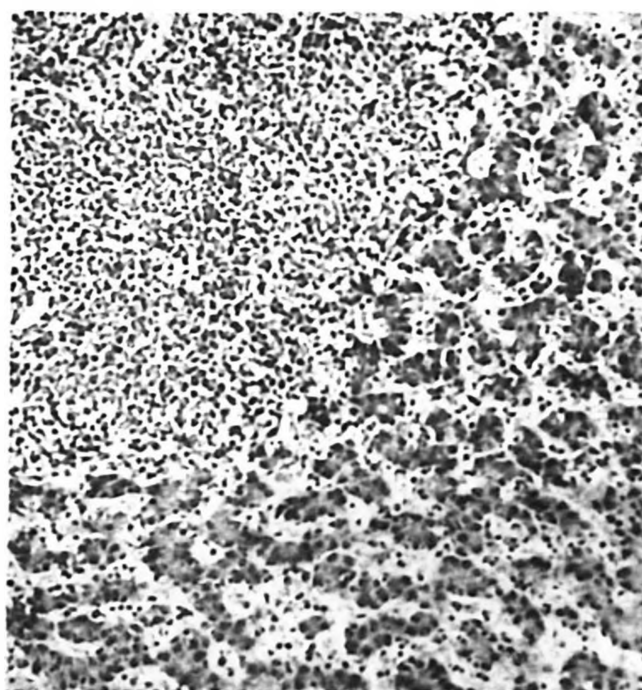
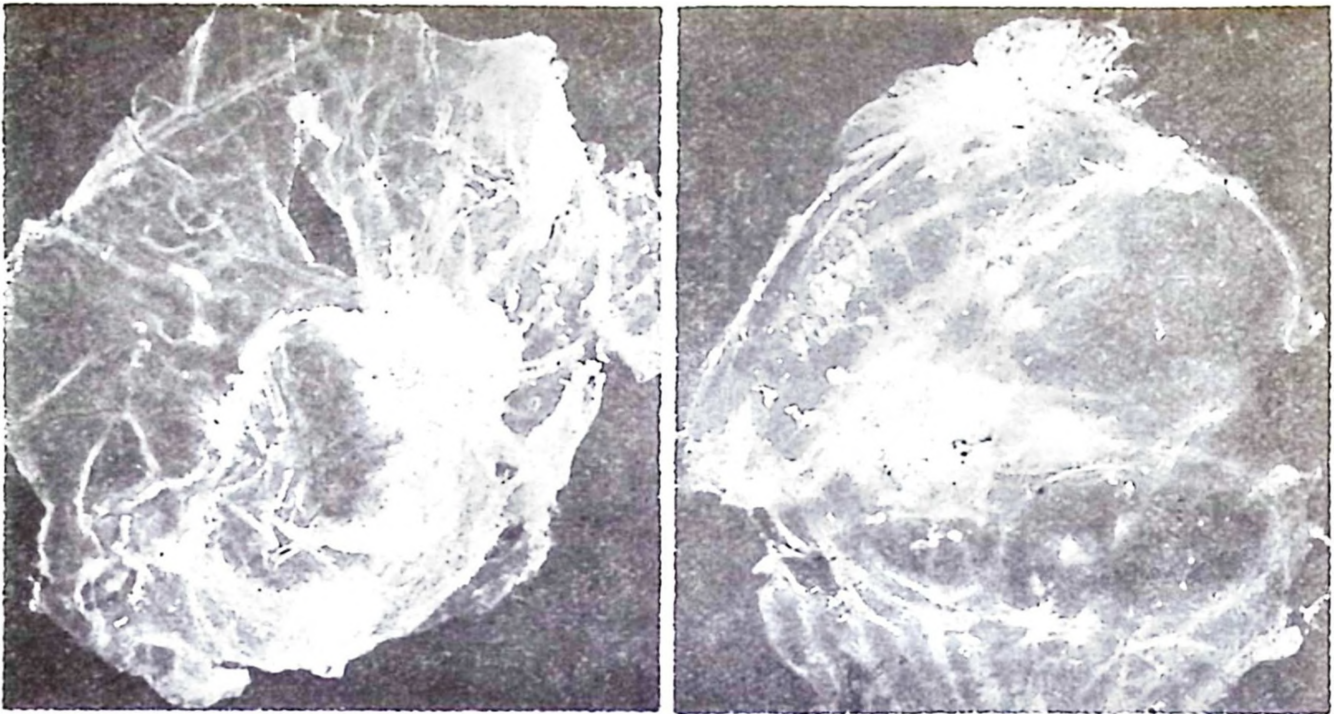


Figure 12. x105. Lymphocytoma in the liver, chicken no. 2.

(Figures 13 and 14). Microscopic examination of the tumor cells showed a variety of cells, some of which had no relation to the original tumor cells. Large polygonal shaped cells were grown from a tumor which was lyomioma-like (Figure 15). A neck tumor (chicken no. 5) produced almost the same kind of cell (Figures 16, 17, and 18) while cells from a kidney tumor (chicken no. 3) produced cells with a perivascular organization (Figure 19).

Tissue culture inoculation : Chicken embryo fibroblasts (CF) were used for tissue culture, and secondary cultures were done in roller tubes and plexiglass plastic petri dishes in a CO₂ incubator.^{3, 4} Plasma samples from chicken no. 3, whose condition was diagnosed as



Figures 13 and 14. Small and large tumors on chorioallantoic membrane produced by the cell-free tumor materials.

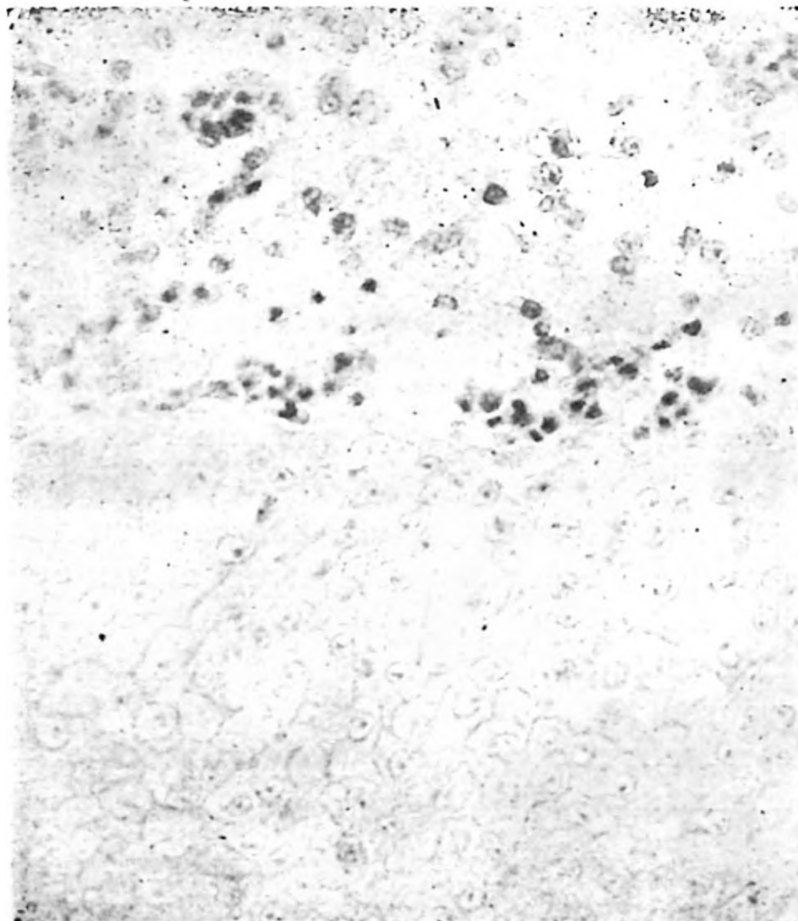


Figure 15. x415. Large polygonal-shaped cells of CAM tumors produced from neck tumor, chicken no. 15.

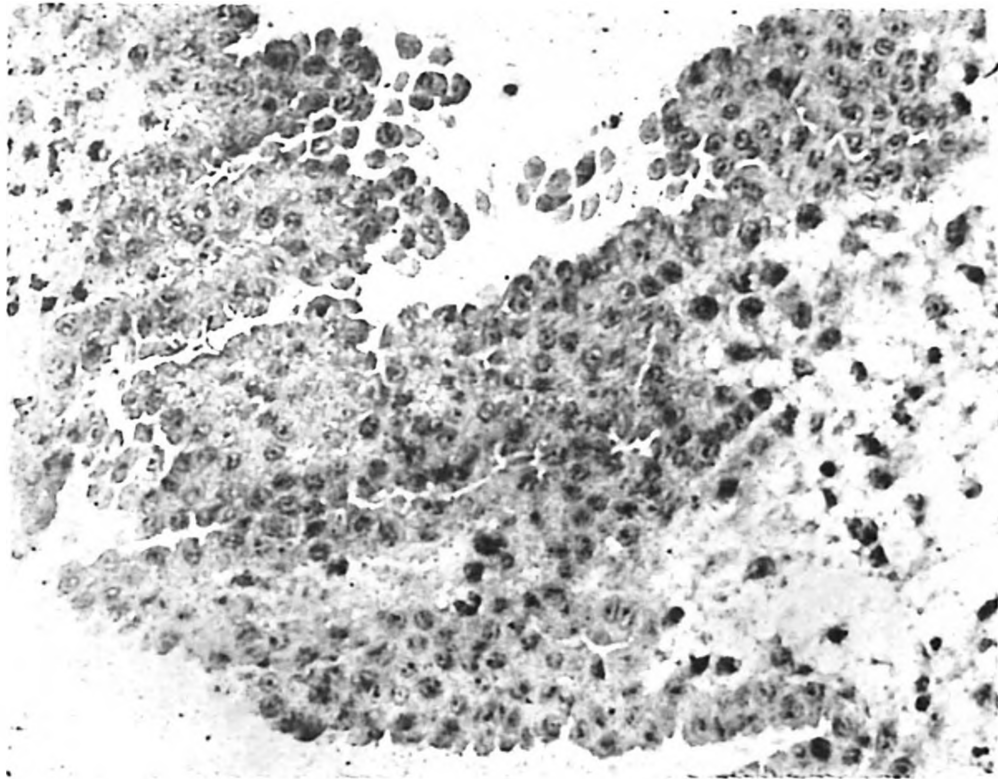


Figure 16. x350. CAM tumor cells produced from mesenteric tumor, chicken no. 5.

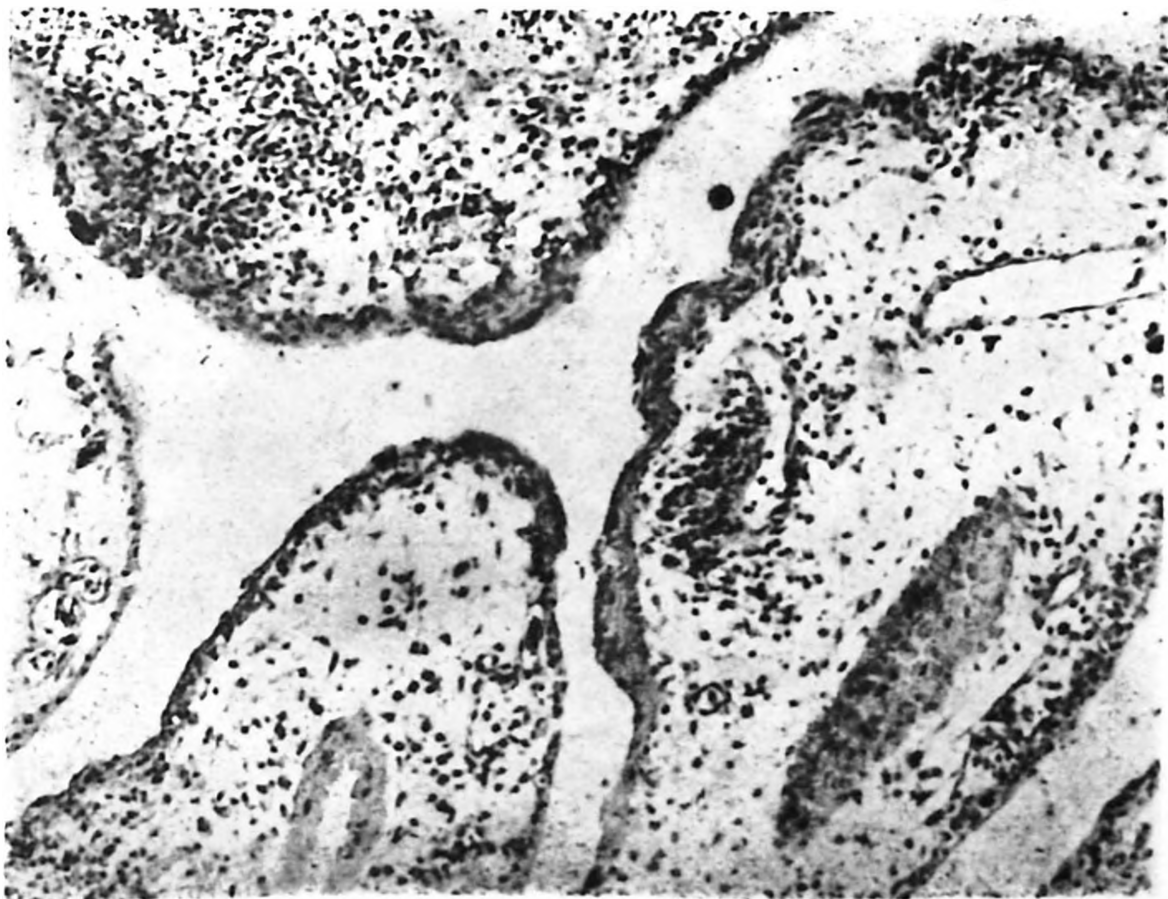


Figure 17. x183. Tumor inoculated CAM showing tumors, chicken no. 5.

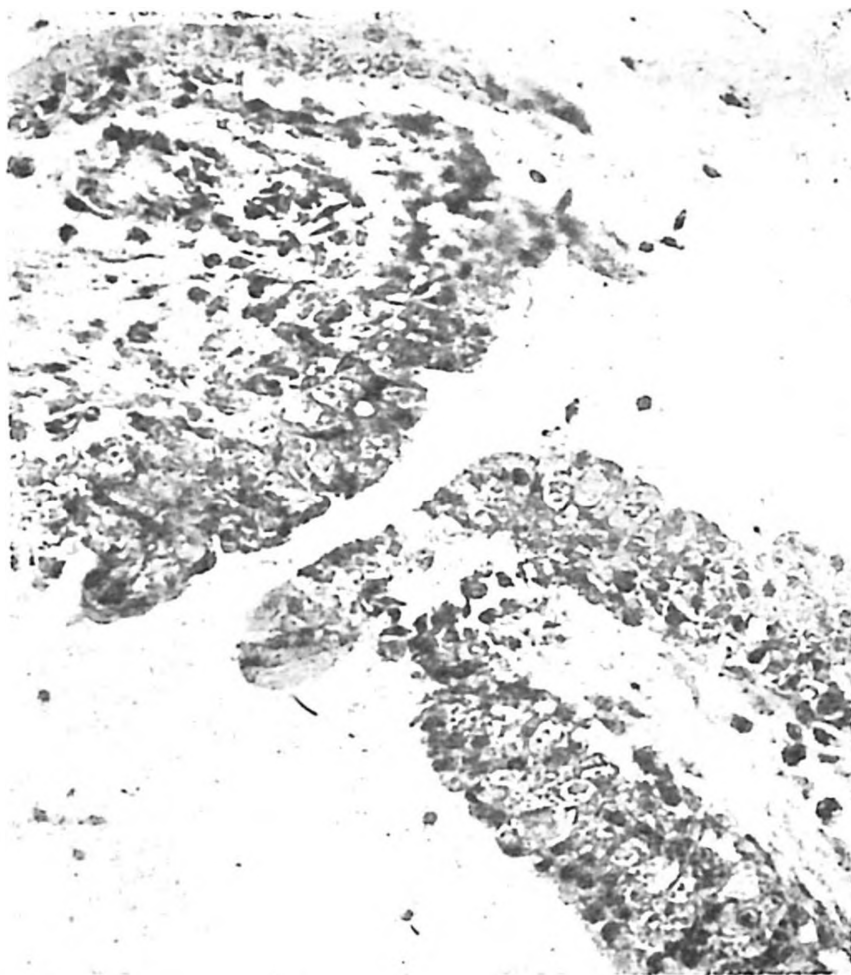


Figure 18. Tumor inoculated CAM showing large rounded cells most of which have inclusions.

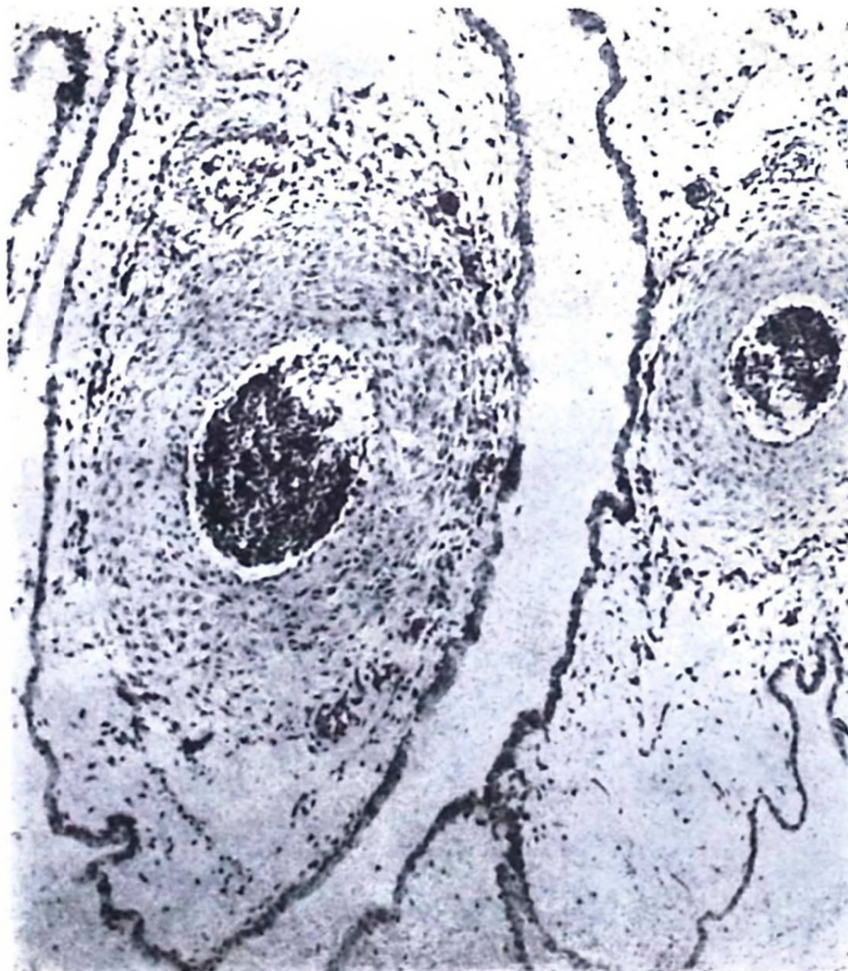


Figure 19. x92. Cell-free kidney material inoculated on CAM showing perivascular cell organization, chicken no. 3.

myeloblastic leukemia, was inoculated as described above, and one week later myeloblastic transformations were observed (Figures 20 and 21). Extracts of the tumors were lytic to chicken fibroblast cells but no such reaction was noted with human cells like WI. 26.³

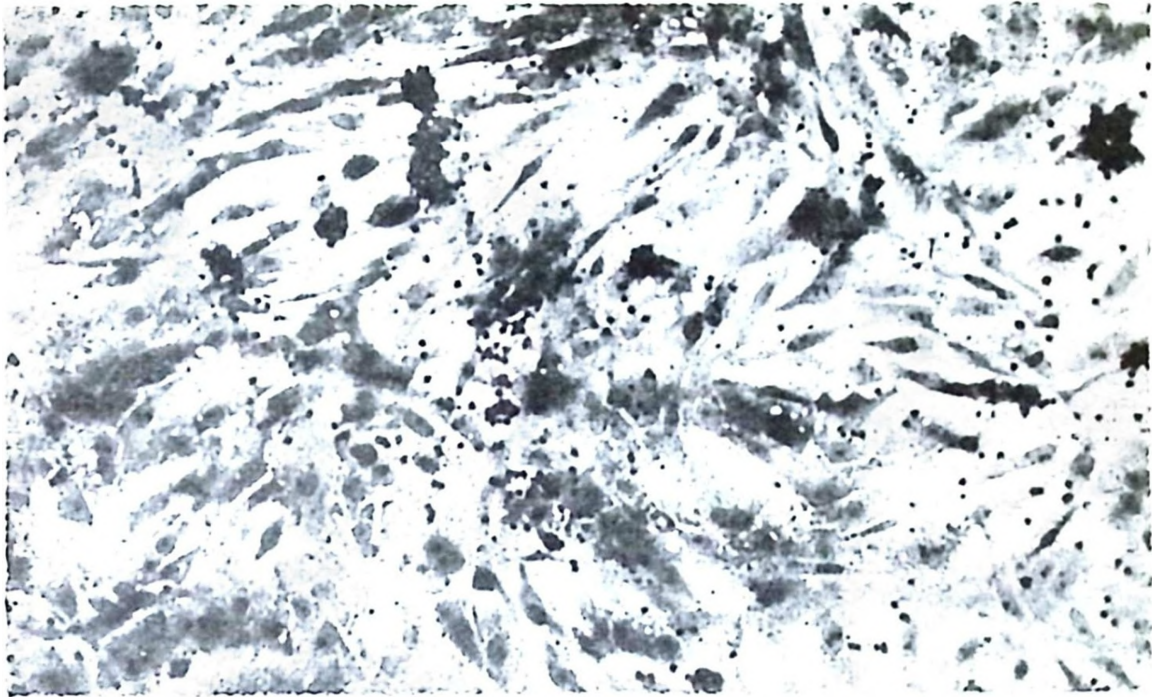


Figure 20. Myeloblastic transformation of CF. T. C.

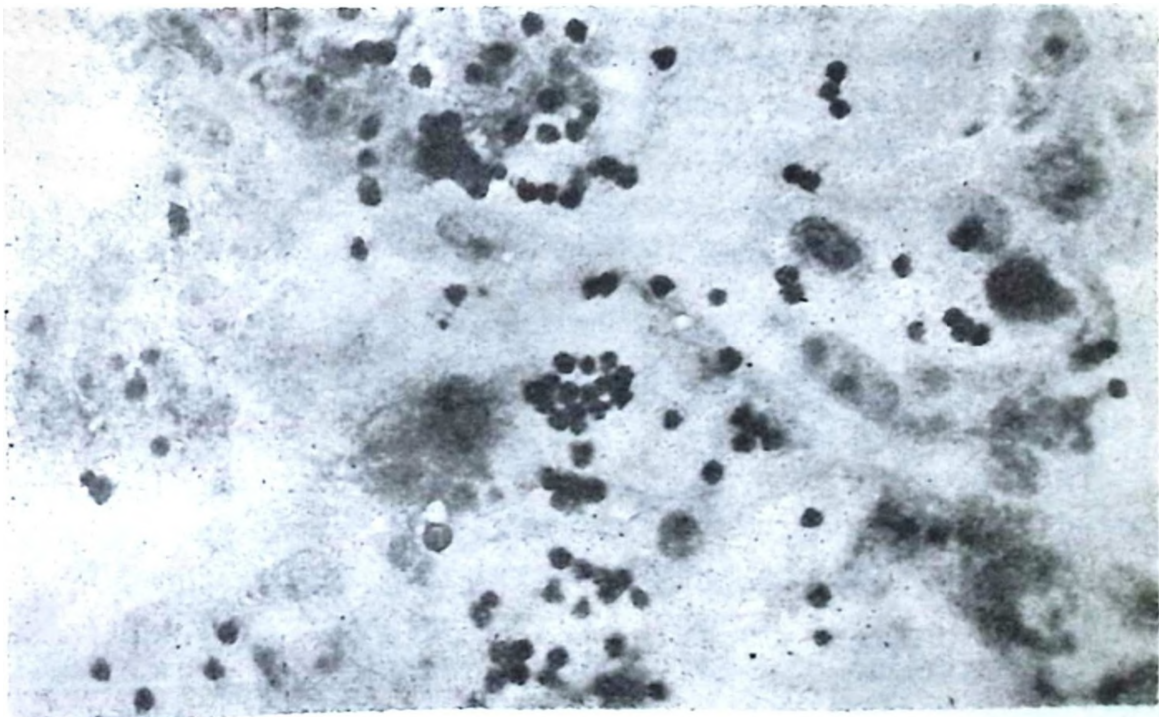


Figure 21. Higher magnification of Figure 20.

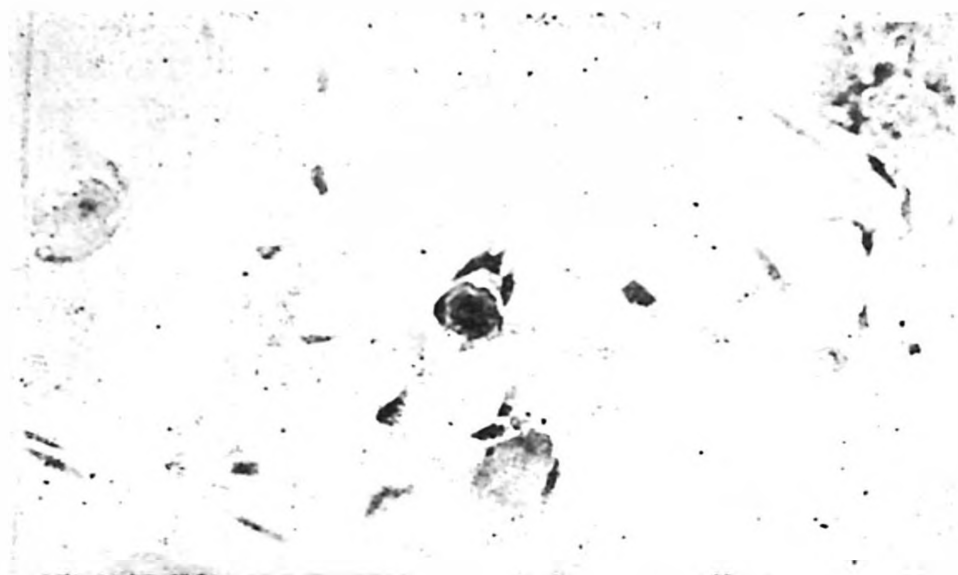


Figure 22. CF. T. C. showing some transformed cells.

Neutralization test : These tests were done on chicken embryo fibroblasts, and the secondary cultures in tubes directly to RSV⁴ instead of Rubin's Rif test,⁶ estimating the focuses produced by RSV on chick fibroblast tissue culture, and neutralization by the sera collected from the stricken hens. Tissue culture must be monolayered, secondary and day-old, otherwise the results are irregular and undependable. Later on this technique was employed on a large scale⁷ and seemed to be a very practical way to demonstrate antibodies against RSV, Bryan and Schmidt-Ruppin (S-R) strains.. Generally, the neutralization titers were higher for the Bryan strain than for the S-R strain (Table 1). Chickens no. 2 and 12 produced no antibodies and were extremely ill and anemic. Chickens no. 6 and 16 produced a high titer of antibodies and had large amounts of acidic fluid and egg accumulation, respectively.

Hamster inoculation : Inoculation of newborn hamsters did not produce any tumors, complement fixation antibodies, or neutralizing antibodies against RSV.

Summary

A natural outbreak among chickens of a highly virulent type produced tumors of different kinds.

The antigenic properties of the tumors were similar to avian leukemia viruses and avian sarcomas of RSV, Bryan and S-R strains.⁸ Sera from the afflicted chickens contained neutralizing antibodies against RSV, Bryan strains, and, to a lesser extent, against S-R strain. Inoculation of CAM produced tumor growth. The solid and organ tumors contained different kinds of cells, including fibrosarcoma, carcinoma, lymphomas and lymphocytomas.

TABLE 1
SERUM NEUTRALIZATION TEST IN CHICK FIBROBLAST TISSUE
CULTURE TUBES

No. of Chick	Neutralization Index Against RSV	
	Bryan Strain	Schmidt- Ruppin Strain
Warrenton 2	0	0
» 3	> 1/80	> 1/40
» 4	> 1/80	> 1/80
» 5	0	0
» 6	< 1/640	< 1/80
» 7	< 1/40	< 1/40
» 8	< 1/20	< 1/10
» 9	< 1/80	< 1/40
» 10	< 1/80	< 1/80
» 11	< 1/40	< 1/20
» 12	0	0
» 13	< 1/80	0
» 14	< 1/40	0
» 15	0	0
» 16	1/320	1/160

REFERENCES

1. Burmester, B.R., and Fredrickson, T.N.: Experimental transmission of avian visceral lymphomatosis and related neoplasms, Colston Papers, Proceedings of the Thirteenth Symposium of the Colston Research Society, University of Bristol, April 10 to 13; 1961, London, Butterworths Scientific Publications, Vol. 13, 1961.
2. Darcel, C.L.: A note on the classification of the leucotic diseases of the fowl, Animal Pathology Division, Canada Department of Agriculture, Veterinary Research Laboratory, Lethbridge, Alberta, 1962.
3. Balluda, M.A., and Goetz, I.E.: Morphological conversion of cell cultures by avian myeloblastosis virus, *Virology* 15:185, 1966.
4. Okuyan, M., and Huebner, R.J.: Unpublished observations.
5. Cook, M.K.: Unpublished observations, National Institute of Allergy and Infectious Diseases.
6. Rubin, H.: A virus in chick embryos which induces resistance in vitro to infection with Rous sarcoma virus, *Proceedings of the National Academy of Science* 46:1105, 1960.
7. Sarma, P.S., Huebner, R.S., and Armstrong, D.: A simplified tissue culture tube neutralization test for Rous sarcoma virus antibodies, *Proc. Soc. Exp. Biol. and Med.* 115:481, 1964.
8. Armstrong, D., Okuyan, M., and Huebner, R.J.: Complement-fixing antigens in tissue cultures of avian leucosis viruses, *Science* 144:1584, 1964.