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Tissue Culture Studies on Relation of Sarcoma to Leukosis of Chickens.*

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These experiments have been undertaken to determine whether sarcoma and leukosis can be produced by a single agent (virus).

Oberling and Guérin¹ described a virus that in their opinion produces leukosis, sarcoma and carcinoma. Troisier² confirmed their observations. Rothe-Meyer and Engelbreth Holm³ and McIntosh⁴ suggested that a single virus may produce both sarcoma and leukosis. During the first half of 1933 we isolated 2 transmissible strains that produce both sarcoma and leukosis and performed numerous experiments to determine whether each of these strains was caused by a mixture of 2 viruses or by a single virus. Some of these experiments are here described.

The strain used in this study (Strain 13, Stubbs and Furth) produces both sarcoma and leukosis. Sarcoma is readily grown *in vitro*, but leukotic blood cells perish within a few days in tissue cultures prepared in the usual manner. Experiments were undertaken to determine whether the ability of this strain to produce erythro-leukosis would vanish with the disappearance of the primitive blood cells from the cultures, but it did not disappear. The cultures remained highly virulent after cultivation *in vitro* for 67 days and produced either sarcoma or sarcoma and erythro-leukosis, as shown in Table 1.

Tissues of a sarcomatous tumor (Strain 13) were incubated *in vitro* in Carrel flasks in the usual manner, in clotted plasma containing approximately 0.5% of embryonic extract, with a supernatant liquid layer of chicken serum diluted with 3 times its volume of Tyrode solution and containing 0.5% of embryonic extract.

Although these sarcoma cells seldom digest the plasma clot, and the addition of foreign tissue is not required to support their growth, nevertheless they are highly virulent for chickens.

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¹ Oberling, Ch., and Guérin, M., *Bull. Assn. franç. étude cancer*, 1933, **22**, 180.

² Troisier, J., *Bull. Assn. franç. étude cancer*, 1934, **23**, 225.

³ Rothe-Meyer, A., and Engelbreth Holm, J., *Acta path. Scand.*, 1933, **10**, 380.

⁴ McIntosh, J., *Brit. J. Exp. Path.*, 1933, **14**, 422.

TABLE I.
Result of Inoculation of Tissue Cultures of Sarcoma 13.

Age of tissue <i>in vitro</i> , days	No. of sub-passage	No. of fowls injected	No. of successful injections	Type of disease produced		Incubation period		Length of life, days
				S*	S and E*	of tumor, days	of blood changes, days	
12	I	4	4	2	2	15 to 25	25	25 to 77
23	III	5	4	2	2	14	28.5	28 to 37
35	III, IV	4	4	4	1	17	30	25 to 34
67	VIII to X	6	5	2	3	17 to 33	32 to 47	37 to 47

* S = sarcoma; E = erythroleukosis.

All chickens of the first 3 groups were Barred Rocks weighing approximately 350 gm.; the chickens of the last group were White Leghorns weighing 750 gm. The incubation periods shown are only approximate. "Length of life" means length of life after inoculation. Most chickens died and a few were killed *in extremis*. Two chickens of the last group are still alive.

The above data indicate that the virus of Strain 13 has multiplied *in vitro* and has retained during 67 days at 39° C. the ability to produce sarcoma or sarcoma and erythroleukosis. Erythroleukosis produced by this strain was associated with diffuse sarcomatosis of the bone marrow and spleen, and it might therefore be regarded as a secondary disturbance due to interference with erythrocyte formation.

The transmission experiments made with this strain are in conformity with this view. Microscopic studies of all instances of erythroleukosis produced by this strain revealed diffuse sarcomatosis of the blood-forming organs, with the exception of 2 cases in which the small sample of the marrow preserved for microscopic study showed only erythroleukosis. The assumption that the agents of sarcoma and leukosis are identical, the route of entry determining the type of disease,¹ is erroneous, for our leukosis Strains 1 and 2 do not produce Rous sarcoma even when introduced by intramuscular injection and the agent of sarcoma, Strain 13, here described produces extensive diffuse sarcomatosis of the spleen, bone marrow, and of several other organs, after intravenous inoculation.

The assumption of recent workers that a single agent may produce in some chickens pure leukosis and in others pure sarcoma or carcinoma requires stronger evidence than hitherto presented. Only one instance of carcinoma was observed in the study of our Strain 13, involving approximately 300 chickens, and all 3 chickens injected with tissues from this carcinoma remained healthy.

Alternative assumptions to the one just proposed are: (a) the

tissue cultures contain 2 viruses (a sarcoma and a leukosis virus) and both grow equally well in the presence of the sarcoma cells; (b) the culture contains one virus that stimulates to neoplastic growth both reticular and endothelial cells, as well as primitive erythroblasts.

Summary. A pure culture of chicken sarcoma is described (Strain 13) that elaborates a highly virulent virus or viruses capable of producing both sarcoma and erythroleukosis.

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Blood Amylase Response to Acetylcholine and its Modification by Physostigmine and Atropine.

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In the course of some experiments on glycogen deposition, it occurred to us that the vagus, which is thought to influence the deposition of glycogen in tissue,¹ might do this through action on the blood amylase. With this in mind, we have studied, in dogs, the variations in blood amylase titre after the intramuscular administration of acetylcholine,* the so-called vagus substance. For the determinations, we have used the viscosimetric method of Elman,² employing the same stock solution of starch for all the determinations in each individual experiment. Determinations were done at intervals varying from 15 minutes to 24 hours. Marked increases in blood amylase values, at times more than 4 fold, were found in 8 experiments on 7 dogs. Table I illustrates these findings.

The fasting blood amylase titre varied between 24 and 60 units, which is in accord with the values found in the literature. For control, determinations were performed on 13 fasting dogs at intervals varying from 15 to 24 hours. It was found that on the same day, using the same stock solution of starch, the variations in the individual dogs did not exceed 3 units. Further, the adminis-

¹ Debois, G., *Compt. rend. Soc. biol.*, 1930, **103**, 546.

* The beta methyl derivative of acetylcholine (Mecholin-Merck) was used throughout these experiments.

² Elman, R., Arneson, N., and Graham, E. A., *Arch. Surg.*, 1929, **19**, 943.