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Further Experiments on the Effect of Testicle Extract on the Agent of Chicken Tumor I.

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Hoffman, Parker, and Walker[1] reported that rabbit testicle extract markedly enhanced the growth of Chicken Tumor I, the effect being the same whether tumor mash or cell-free filtrates of the tumor were used in the inoculations. Sturm and one of us[2] could not duplicate these findings despite the use of a variety of both testicle extracts and active tumor preparations.

Since more work was indispensable in order to elucidate the cause of the discrepancies we first resorted to the technique of progressive dilution of the active tumor material. Accordingly, water dilutions of the fresh tumor tissue, ranging from 1:6 to 1:10,000 were obtained and 0.5 cc. of each dilution was injected with its volume of filtered bull testicle extract diluted 1:2. Each chicken was inoculated intradermally in 2 or 3 areas in each side of the

TABLE I.

No. of tests	Dilution of tumor extracts	Aver. area of tumor surface after 14 days		Aver. area of tumor surface after 21 days	
		Testicle extract	H_2O	Testicle extract	H_2O
		sq. cm.	sq. cm.	sq. cm.	sq. cm.
9	1:6	6.2	4.8	12.0	8.7
8	1:60	4.0	4.1	6.1	9.1
8	1:300	1.6	1.1	4.7	3.4
3	1:600	4.5	.1	.5	.0
2	1:3,000	.05	.0	.1	.0
1	1:12,000	.0	.0	3.0	.0
1	1:30,000	.0	.0	2.0	.0
1	1:60,000	.0	.0	.0	.0

[1] Hoffman, D. C., Parker, F., and Walker, T. T., *Am. J. Path.*, 1931, **7**, 523.

[2] Sturm, E., and Duran-Reynals, *J. Exp. Med.*, 1932, **56**, 711.

breast. The customary spreading was noticed after each injection. The results are summarized in Table I.

Table I shows some enhancement by testicle extract, but far less regular and marked than in the case of viruses.

In the foregoing tests, the mixture was inoculated intradermally at points located on both sides of the median breast line. With the bird in normal position this region is more or less horizontal and occupies the lowest part of the body. It is obvious that in this case the mixture injected had little opportunity to spread. Under special conditions the spreading agent may even drain back the active material. This fact may well account for the observation that in certain cases tumors obtained from testicle extract mixtures were smaller than the controls.

In the following experiments, the material was inoculated into the skin, either in a lateral region under the wing, or in the upper part of the leg. The active material was obtained by extracting fresh chicken tumor pulp with 12 volumes of sterile distilled water. The fluid after centrifugation was used for the test and mixed before inoculation with an equal volume of fresh rat testicle extract. The latter had been prepared in the usual manner by extracting with an equal volume of water, and using the supernatant fluid from centrifugation.

As illustrated in Table II, the results were conclusive and the tumors obtained were incomparably larger than in the case of the control injection, simply diluted with an equal volume of Ringer's solution.

TABLE II.

Exp. No.	No. of tests	Amt. of mixture injected	Aver. size of main tumor surface after 18 days		Aver. area of skin with scattered nodules around the main tumor	
			Testicle extract	Ringer's solution	Testicle extract	Ringer's solution
		cc.	sq. cm.	sq. cm.	sq. cm.	sq. cm.
1	1	.5	32.8	8.6	51.1	.0
2	1	.5	12.0	8.6	36.0	.0
3	2	.8	26.7	3.7	59.0	.0
4	2	.8	19.5	3.7	40.8	.0
5	3	.6	61.2	7.0	.0	.0
6	3	.6	46.0	6.3	.0	.0
7	1	.6	32.0	5.0	.0	.0
Average surface of tumor			34.0	6.1	48.4	.0

Conclusion. In agreement with the results of Hoffman, Parker, and Walker, Chicken Tumor I agent is spread when injected together with testicle extract and the resultant lesions are markedly enhanced.