

PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN
TUMOR

VIII. THE EFFECT OF TESTICLE EXTRACT ON THE RATE OF GROWTH
OF CHICKEN TUMOR I*

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The observation that testicle extract markedly enhances the infectivity of certain viruses and bacteria, reported by one of us (Duran-Reynals (1)) has been confirmed and extended by a number of investigators (2). It has been further shown that testicle extract exerts an inhibitory influence on the growth of a transplantable epithelioma of the rabbit (3) and to a less extent on a sarcoma of the mouse (4). It is of interest to know what effect testicle extracts will have on the chicken neoplasms, transmissible by cell-free extracts.

Recently Hoffman, Parker and Walker (5) have published the results of a similar investigation in which they report evidence of the enhancement of the chicken tumor agent by rabbit testicle extract, but none by that from rooster testicle. This evidence is based on two positive experiments, but the same testicle extract was used in both tests. They draw no conclusions as to the nature of the tumor agent from these results, but give the impression that further investigation with the method might lead to some conclusions on the question. During the last 3 or 4 years a considerable amount of data had been accumulated in this laboratory, but had not been published at the time the article referred to appeared. Since our results differed from those reported by the above authors, we deferred publication until additional tests could be made with certain minor modifications in technique suggested by their paper.

* This investigation was carried out by means of the Rutherford Donation.

Materials and Methods

The testicle extracts were prepared by removing the glands of rat, rabbit or rooster, trimming them of all adhering tissue and removing the capsule. The tissue was then ground with sand in a mortar along with an equal volume of physiological salt or Ringer's solution. After centrifuging down the heavier particles, the supernatant fluid was decanted, filtered through a Berkefeld V candle and used in the tests.

The testicle extracts of rat, rabbit and rooster and the purified bull testicle extract were tested with (1) the active extract of desiccated Chicken Tumor I, (2) the concentrated Berkefeld filtrate of fresh Chicken Tumor I and (3) suspensions of the tumor cells.

The Effect of Testicle Extract on the Activity of Extracts of Chicken Tumor I Desiccates

These experiments, both in the method of preparation of the testicle extracts and the location of inoculations, followed closely the technique used in testing the action of the enhancement factor on viruses and bacteria which has been reported by one of us (Duran-Reynals).

Experiments.—The tumor extract was prepared by thoroughly extracting 1 gm. of finely powdered desiccate of Chicken Tumor I with 50 cc. of distilled water and adding enough N/10 NaOH to render the solution either neutral or slightly alkaline. A pH of 7.2 to 7.4 was usually maintained in all extractions. The emulsion suspension was thoroughly mixed by passing it in and out of a large hypodermic syringe fitted with a 12 gauge lumbar puncture needle for several minutes. After centrifugation the supernatant fluid was filtered through moistened, coarse filter paper and divided into 5 portions. To each of the first 4 portions was added an equivalent amount of testicle extract to be tested, while to the remaining 5th portion an equal volume of either salt or Ringer's solution was added—the latter to serve for the activity control injections. In some of the tests the testicle extracts were diluted 10 times with Ringer's solution, but this modification of the method had no effect on the results. In consequence all of the experiments are grouped together. The solutions of the testicle and chicken tumor extracts were thoroughly mixed and allowed to stand at room temperature for the length of time necessary to complete the injection. This period usually varied from 20 to 40 minutes. 0.4 cc. of each of the respective mixtures, including the control, was injected intradermally into a normal chicken. By this means each chicken received the 4 test injections and the control for activity. It was noted that the bleb resulting from the intradermal injection of the solutions containing testicle extract flattened in a very few minutes after injection, while those without testicle extract required considerably longer to spread into the surrounding tissues.

Weekly measurements of the resulting tumors were made. The figures on the

size of the tumors recorded for each chicken were the last before the tumors broke through into the subcutaneous tissue or coalesced in the skin. This method in our opinion gives a fair picture of the results. As each bird carried all of the test inoculations as well as the control injections, the variation in susceptibility of individual fowls and in the activity of the different tumor extracts is minimized. The chickens used in the tests were of the Plymouth Rock type, adults of uncertain age, bought in the open market. Fresh extracts were used in each group. The outcome of 4 experiments, based on 72 inoculations, is given in Table I.

There is no evidence of enhancement of the chicken tumor agent by the various testicle extracts with the methods used in these experiments. Nor do we find any inhibiting action, with the possible exception of the tests with purified bull testicle; but the numbers here are too small to be significant. Furthermore, at no time during the course of the experiments was there any material evidence of enhancement or inhibition in the development of the tumors.

TABLE I
The Action of Testicle Extract on Chicken Tumor I Extracts
(4 experiments)

Chicken Tumor I extract	No. of inoculations	No. of tumors	Average size of tumors
			<i>cm.</i>
Rat testicle extract.....	20	20	2.5 x 2.0
Rabbit testicle extract.....	8	8	2.3 x 1.8
Rooster testicle extract.....	16	16	2.5 x 2.0
Purified bull testicle extract.....	4	4	1.6 x 1.1
Salt solution (control).....	24	24	2.3 x 1.9

The Effect of Testicle Extract on the Tumor Production by a Concentrated Berkefeld Filtrate of Chicken Tumor I

As a further test of the possible effect of testicle extract on the chicken tumor agent, filtrates of fresh tumor extracts were utilized in the next group of experiments instead of the extracts of tumor desiccate, which were the source of the agent in the preceding group.

Experiments.—Berkefeld filtrates were prepared by grinding 25 gm. of mashed chicken tumor tissue in a mortar along with a quantity of sterile sand, diluting with 500 cc. of distilled water and adding N/10 NaOH to bring the pH of the solution to 7.2–7.4. The solution was shaken for 20 minutes in a stoppered flask, centri-

fuged and the supernatant fluid passed through Berkefeld V candles under 25 lbs. of air pressure. 300 cc. of the filtrate was collected and concentrated to 40 cc. in alundum thimbles lined with 8 per cent collodion membranes. The testicle extracts were prepared in the same manner as in the previous experiments.

For the test, 0.4 cc. of mixtures of equal parts of the concentrated filtrate and testicle extracts was injected intradermally in chickens. For the control of activity an equal amount of the concentrate diluted with Ringer's solution was injected into each chicken. Fresh testicle extracts and tumor filtrates were prepared for every experiment. The results of two groups of tests are given in Table II.

While the number of fowls inoculated in this group is not large, yet they show a uniform lack of any effect of the testicle extracts on the size of tumors produced by tumor filtrates.

TABLE II
*The Action of Testicle Extract on Fresh Concentrated Berkefeld Filtrate of
Chicken Tumor I*
(2 experiments)

Concentrated Berkefeld filtrate of Chicken Tumor I	No. of inoculations	No. of tumors	Average size of tumors
			<i>cm.</i>
Rat testicle extract.....	6	6	1.8 x 1.3
Rooster testicle extract.....	6	6	1.8 x 1.6
Salt solution (control).....	6	6	2.1 x 1.5

*The Effect of Testicle Extract on the Size of Tumors Resulting from the
Inoculation of Tumor Cells*

As stated above, testicle extract definitely inhibits the growth of a transplantable rabbit tumor and to a less extent of a certain transplantable mouse tumor. In these instances the tumors are transferred only by cells. To parallel these experiments more closely we have tested the effect of testicle extract on the size of tumors produced by the chicken tumor cells.

Experiments.—The chicken tumor cell suspensions were made by taking 1 cc. of freshly mashed chicken tumor tissue, passing it through a fine wire gauze mesh under slight pressure and suspending the cells in 5 cc. of Ringer's solution. For the injection a mixture was made of equal parts of the cell suspension and the respective testicle extracts. The total volume injected into each area of a normal chicken was 0.4 cc. The results of these experiments are shown in Table III.

From the figures given in Table III there seems to be no enhancement of the growth rate of tumors resulting from mixtures of chicken tumor cells with testicle extract from four different species.

The Effect of Testicle Extract on the Development of Tumors in Young Fowls

Hoffman, Parker and Walker (5), in their experiments referred to above, used 8 weeks old chicks and reported enhancement with extracts of rabbit testicle (2 experiments with the same extract), but none with that from the rooster. In order to check the possibility that the age of the fowl affects the results our experiments have been repeated using chicks 6 to 8 weeks old.

TABLE III
The Action of Testicle Extract on Chicken Tumor I Cells
(3 experiments)

Chicken Tumor I cell suspension	No. of inoculations	No. of tumors	Average size of tumors
			<i>cm.</i>
Rat testicle extract.....	6	6	2.0 x 1.4
Rabbit testicle extract.....	14	14	2.2 x 1.6
Rooster testicle extract.....	3	3	2.0 x 1.4
Purified bull testicle extract.....	2	2	1.9 x 1.5
Salt solution (control).....	12	12	2.3 x 1.5

Experiments.—The tests were confined to the effect of rat and rabbit testicle extracts, but these were tested on both tumor cells and extracts of desiccated tumor, the inoculations being made intradermally. The conditions of the experiments were the same as in the preceding groups, except that young chicks were used. The results of these experiments, with fresh extracts for each experiment, are given in Table IV.

A further experiment was carried out with 8 weeks old chicks, in which a mixture of chicken tumor cells and rat testicle extract was injected intramuscularly in one side, and in the other the tumor cell suspension appropriately diluted for control. The measurements of the resulting tumors suggested some enhancement in 3 of the 4 fowls. They were killed at the height of the experiment and the true measurements made at autopsy showed no material difference between the two sides. This was verified by the weight of the two tumors in the one chick showing the greatest difference in the crude measurements.

From these last tests it seems that there is no evidence of enhancement of tumors by testicle extract in young chicks, thus confirming the results we had obtained in adult birds.

TABLE IV
The Action of Testicle Extract on Chicken Tumor I
8 Weeks Old Chicks Injected
(3 experiments)

Material injected	No. of inoculations	No. of tumors	Average size of tumors
			<i>cm.</i>
Chicken tumor cells with:			
Rat testicle extract.....	4	4	3.3 x 2.5
Rabbit testicle extract.....	4	4	3.2 x 1.9
Salt solution (control).....	8	8	3.2 x 2.3
Chicken tumor extract with:			
Rat testicle extract.....	7	7	2.3 x 2.2
Rabbit testicle extract.....	7	7	2.1 x 1.6
Salt solution (control).....	14	14	2.2 x 1.7

DISCUSSION

With testicle extract we have a factor or factors which augment the infection of viruses and bacteria on the one hand, and on the other seem to retard the growth of certain transplantable tumors. With this evidence it might be supposed that the action of testicle extract on the chicken tumor would give suggestive information as to the nature of the causative agent. However, since the testicle factor enhances infective agents (6) by increasing tissue and cell permeability, and since it enhances the action of certain toxins (7), it would appear that no deduction could have been drawn as to the nature of the chicken tumor agents even if the experiments had shown a definite enhancement. As a matter of fact we have found no evidence of definite enhancement or inhibition with the various testicle extracts investigated. There may be some simple explanation in this failure such as the structure of the fowl's skin, but this does not seem likely since the ability of the testicle extract to bring about an increase in the area of spread of injected materials is just as evident in the chicken

skin as in that of the mammal. We see no immediate explanation of the difference in the results of our experiments and those of Hoffman, Parker and Walker.

SUMMARY

Extracts prepared from the testicle tissue of the rat, rabbit, fowl or bull, injected together with extracts of Chicken Tumor I or with cells of this tumor, showed no definite effect of either enhancement or inhibition as concerned the resulting tumors.

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