PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR

VI. Action of the Associated Inhibitor on Mouse Tumors*

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In previous communications evidence has been presented which indicates that an inhibitor is present in transplantable chicken tumors, together with the tumor-producing agent (1). The bases of the assumption that such an inhibitor exists are first, that the removal of a fraction from a tumor extract leaves the tumor agent in a far more active form, and second, that the inhibitor can be extracted from certain tumors in sufficient concentration to neutralize the tumor-producing property of the most active tumor extracts. It has been suggested that the two factors present in the chicken tumor, an agent which causes the malignant transformation of cells and an inhibitor which tends to balance or neutralize this agent, are related to the factors which control the growth and differentiation of normal tissues (2). The tumor agent (Chicken Tumor I) when first studied exhibited a pronounced degree of species specificity, but now shows it to a less extent. On the other hand, many active cell products are not limited in their action to the species producing them. On the basis that the inhibitor from the chicken tumor might be less limited in its effect than the agent, it has been tested on mouse tumors. The results are given in the present paper.[1]

Methods and Materials.—The following materials known to neutralize or inhibit the chicken tumor agent were tested on mouse tumors: extracts of desiccated slow-

*This investigation was carried out under the Rutherford Donation.

[1] A preliminary note on this work has been published (Murphy, Jas. B., and Sturm, E., *Science*, 1931, **74**, 180).

growing chicken tumor,[2] exudates from slow-growing tumors, sera from immune chickens, and sera from immunized rabbits (3). As controls to the above tests the following materials, known not to affect the chicken tumor agent, were investigated: extracts of desiccated rapidly growing chicken tumor, exudates from rapidly growing tumor, muscle, brain, and liver from normal chickens, muscle from immune chickens, and normal rabbit and chicken sera. The test solutions were prepared by thoroughly extracting 1 gm. of the tissue desiccates with 30 cc. of water, maintaining the pH at about 7 by the addition of N/10 NaOH. The extracts were then centrifuged to remove the larger particles and the supernatant fluid heated at 52°C. for 30 minutes. The latter procedure was used to destroy the tumor agent in the active extracts and for uniformity the treatment was carried out on all the controls.

The transplantable mouse carcinoma utilized in the experiment was a standard tumor, known as Bashford 63. It usually gives a fairly high percentage of takes and does not often retrogress when once established. The sarcoma principally used is also a standard tumor, known as Crocker 180, characterized by the high percentage of takes it gives in practically all strains of mice, and by the fact that it is not easily influenced by procedures which increase animals' resistance to many of the other transplantable tumors. A third tumor, Mouse Sarcoma S/37, had its origin in the stroma of a transplantable adenocarcinoma, and is notable for its rapidity of growth.

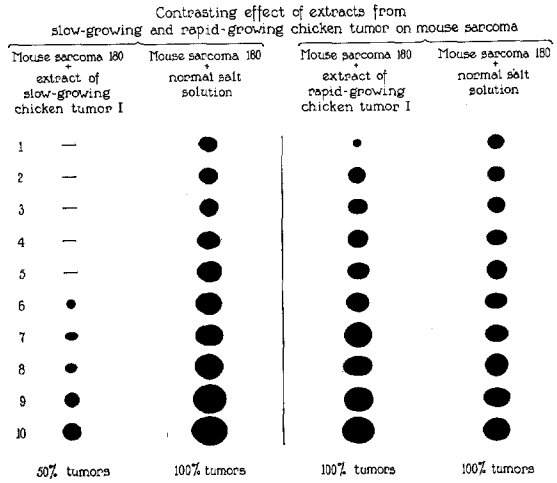
In the test with the carcinoma, grafts of the usual size were cut from the solid part of young tumors; these were placed in the extracts and nicked in several places to give a greater surface of exposure. The controls were immersed in salt solution. The contact was only for the time required to load the grafts into trocars for inoculation. With the sarcomas a suspension was made by forcing the tumors through a fine grill and adding 3 times the volume of normal salt solution. Part of this suspension was mixed with equal amounts of the test extract or fluid and 0.1 cc. inoculated into mice. For the controls the suspensions were diluted with salt solution and equal amounts inoculated. In practically all of the experiments the mice received the test inoculation in one groin and the control in the other, with additional animals inoculated with the control alone.

The Effect of Extracts of Chicken Tumor I on Transplantable Mouse Tumors

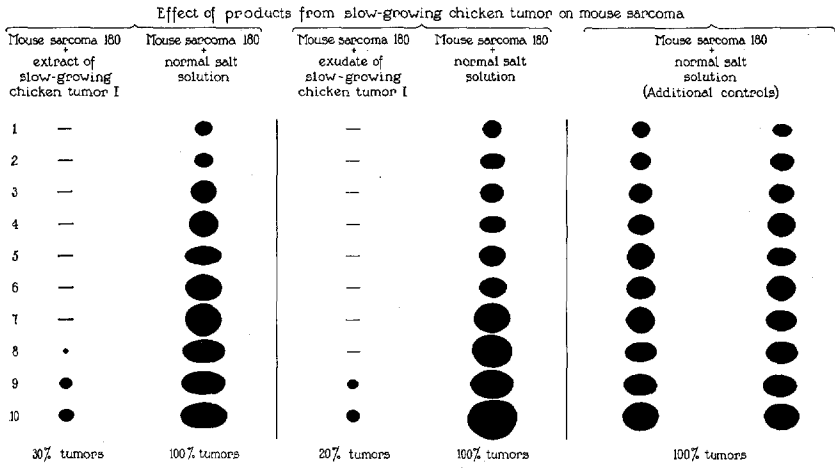
The action of chicken tumor extracts such as are known to inhibit the chicken tumor, and of others without this effect have been princi-

[2] The fact should be emphasized that not all slow-growing examples of Chicken Tumor I yield sufficient inhibitor to have the marked effect reported in this and previous papers. Extracts of desiccates of a large number of tumors were tested and those yielding the greatest concentration of inhibitor were utilized in this test. It is possible that the slow growth rate of some tumors depends on factors other than the presence of an inhibitor in the tumor.

pally tested. So far the investigations have been confined to experiments with the three mouse tumors, for it seemed more important at



TEXT-FIG. 1



TEXT-FIG. 2

the moment to multiply the control tests than to extend the observations to a larger variety of tumors.

Experiments.—The average individual experiment was made up of 30 mice divided into groups of 10. Two of these received inoculations of tumor plus a test

fluid in one groin and in the other a control inoculation of the tumor with normal salt solution. The third group was inoculated in both groins with the tumor in

TABLE I

Experiment number	Material inoculated	Number of inoculations	Number negative	Negative	χ^2	P
				per cent		
1	Mouse Tumor 180 plus extract slow C.T.I.	131	102	77.9	126.0	0.000,000
	Mouse Tumor 180 plus salt solution	163	21	12.9		
2	Mouse Tumor 180 plus extract rapid C.T.I.	20	2	10.0	0.6	0.4
	Mouse Tumor 180 plus salt solution	60	3	5.0		
3	Mouse Tumor 180 plus exudate slow C.T.I.	18	16	88.9	61.7	0.000,000
	Mouse Tumor 180 plus salt solution	54	0	0.0		
4	Mouse Tumor 180 plus exudate rapid C.T.I.	20	0	0.0	1.0	0.3
	Mouse Tumor 180 plus salt solution	60	3	5.0		
5	Mouse Tumor 63 plus extract slow C.T.I.	46	10	21.7	0.0	1.0
	Mouse Tumor 63 plus salt solution	87	19	21.8		
6	Mouse Tumor 63 plus exudate slow C.T.I.	17	2	11.8	0.7	0.4
	Mouse Tumor 63 plus salt solution	38	8	21.1		
7	Mouse Tumor 180 plus boiled extract slow C.T.I.	39	11	28.2	0.2	0.65
	Mouse Tumor 180 plus salt solution	50	12	24.0		

salt solution. This use of double controls was done to detect a possible general effect from the local injection of inhibitors. As there was no indication of such

action, the results of the control inoculations, whether in the test animals or in those receiving only control inoculations, are grouped together. In several experiments with materials which failed to show any inhibiting action in two tests the results of the 18 or 20 inoculations were considered sufficient.

The results of two individual experiments are given in Text-figs. 1 and 2. The first contrasts the action on Mouse Tumor 180 of a chicken tumor extract known to inhibit chicken tumors with one

TABLE II

	Number of inoculations	Number negative	Negative
			per cent
Mouse Tumor 180 plus extract normal muscle..	19	1	5.3
Mouse Tumor 180 plus salt solution.....	32	4	12.5
Mouse Tumor 180 plus immune chicken muscle extract.....	9	1	11.1
Mouse Tumor 180 plus salt solution.....	22	1	4.5
Mouse Tumor 180 plus normal chicken serum..	29	3	10.3
Mouse Tumor 180 plus salt solution.....	60	4	6.7
Mouse Tumor 180 plus immune chicken serum.	19	0	0.0
Mouse Tumor 180 plus salt solution.....	40	1	2.5
Mouse Tumor 180 plus normal rabbit serum...	20	0	0.0
Mouse Tumor 180 plus salt solution.....	40	1	2.5
Mouse Tumor 180 plus immune rabbit serum..	20	0	0.0
Mouse Tumor 180 plus salt solution.....	40	1	2.5

which had no such effect. The second shows the inhibiting action of an extract and an exudate from a slow-growing chicken tumor. The data from all of the experiments, based on over 1000 inoculations, have been brought together in Tables I and II.

In addition to the figures for the tests and controls of each group, we have included an analysis of the principal experiments (Table I) by applying the χ^2 test with its corresponding probabilities (4). This method tests the independence of the proportionate differences between the two groups under comparison. P is a measure on the scale of 0 to

1 of the probability that the deviations from the theoretical frequencies may be reasonably supposed to be due to the errors of sampling. If P is below 0.02 we may consider that a real effect had been produced. It will be noted that Experiments 1 and 3 in Table I show an unquestionable difference between the test and the control inoculations, which may be considered proof of an inhibiting action of the extracts and exudates of slow-growing tumors. In Experiments 2 and 4, where the extracts and exudates were obtained from rapidly growing chicken tumors, no inhibiting action is indicated. In Experiment 5 the unusual value of $P = 1$ was obtained, but undoubtedly the extract in this case had no effect on the Tumor 63. The destruction of the inhibitor by boiling is shown by the results in Group 7.

Table II lists a number of experiments based on smaller numbers, in which tests with extracts from muscle and with normal and immune sera gave negative results.

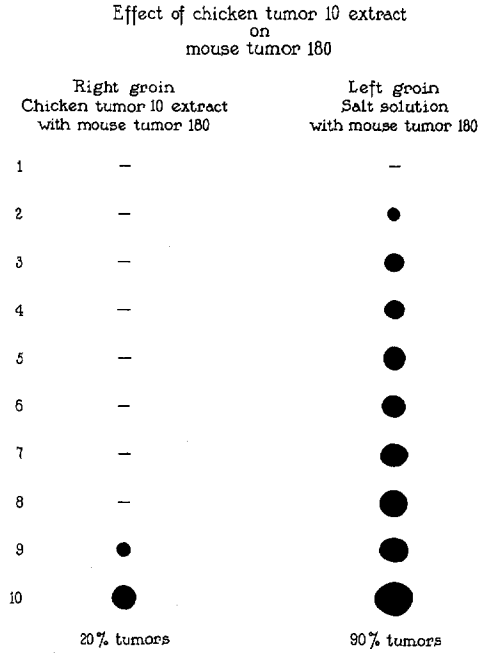
In addition to the experiments included in the tables a few tests were made with extracts of desiccated brain and liver of normal chickens, which were found to be without effect on Mouse Tumor 180. Extensive tests with Mouse Tumor S/37 failed to show any influence on its growth after treatment with extracts known to inhibit chicken tumors and Mouse Tumor 180.

The number of tests is sufficiently large to leave little doubt that the extracts of certain relatively slow-growing chicken tumors and the exudates from such tumors have a definite inhibiting action on a transplantable mouse sarcoma and are without effect on a mouse carcinoma. The number and variety of the controls very largely eliminate the possibility that the result is due to injury from some incidental enzyme or chemical. Perhaps the best indication of this is the failure of products of the rapidly growing tumors to exert any effect.

Effect of Products of Chicken Tumor X on Mouse Tumor

Andrewes (5) has reported that the serum from chickens bearing either of two slow-growing fibrosarcomas for at least 5 months will neutralize the tumor agents of Chicken Tumor I and the tumor known as Mill Hill 2. He considers this property in the nature of a virus antibody, which would indicate a common or closely related etiologic agent for these tumors. The preceding experiments show that the anti-

bodies developed against the tumor agent either in chickens showing a certain amount of natural resistance or in rabbits actively immunized, while capable of neutralizing the chicken tumor agent, are without effect on the mouse tumors. The possibility that the neutralizing property of the sera described by Andrewes represents the action of an inhibiting factor instead of an antibody has not been eliminated. The following experiments with Chicken Tumor X probably throw some light on the question, as the sera from fowls bearing this tumor were used by Andrewes in his experiments referred to above.



TEXT-FIG. 3

Experiment.—Chicken Tumor X has been used as the source of extracts. This tumor, a transplantable fibrosarcoma, derived from a spontaneous tumor, has been under investigation in this laboratory for the last 5 years. As a rule it grows very slowly, often requiring from 8 months to over a year to kill the animal. During this period it attains enormous size. At times it has grown more rapidly but even at these periods metastases have taken place with great rarity. It is transmitted with difficulty by desiccates and only one doubtful result has been obtained with filtrates.

The methods used were the same as those for the preceding group of experiments. The desiccates were prepared from tumors of about a year's growth and the extracts tested on Mouse Tumor 180. The results are presented in Table III and an individual experiment in Text-fig. 3.

It is evident from the figures in Table III, based on five experiments in which 138 inoculations were made, that Chicken Tumor X yields an inhibitor for Mouse Tumor 180. The percentages of complete inhibition are not as striking as those with the inhibitor from Chicken Tumor I. It might have been expected that the inhibitor from the former tumor would be less potent, as it is associated with a tumor agent of relatively low grade activity.

TABLE III

	Number of experiments	Number of inoculations	Number negative	Negative
				per cent
Mouse Tumor 180 plus extract of Chicken Tumor X	5	69	41	59.5
Mouse Tumor 180 plus salt solution	5	69	4	5.8

DISCUSSION

The principal question suggested by the findings is whether the inhibiting action exerted by the extracts of certain desiccated chicken tumors represents a definite, specific force or whether it represents an incidental result, devoid of importance. It is difficult to reconcile the lack of an inhibiting element in extracts from rapidly growing tumors with the latter view. While as yet sufficient evidence is not available for a final conclusion, there are certain facts which justify a tentative interpretation. Perhaps the most important of these is that the inhibitor from a chicken sarcoma acts on a mouse sarcoma and not on a mouse carcinoma; but this observation must be extended to a large variety of tumors before we can accept the reaction as specific. The absence of demonstrable effect of the inhibitor on another mouse sarcoma (S/37) may be due to the unusual malignancy of this tumor, or there may be some question as to the nature of this growth which is supposed to be a sarcoma but had its origin in the stroma of a carcinoma.

If the inhibitor is a definite factor its possible relationship to anti-

bodies must be considered. The fact that antibodies developed against the chicken tumor have no effect on the mouse sarcoma while inhibitor derived from the tumor does retard these growths, suggests a difference. Andrewes (5) has expressed some doubt that a substance identical with the serum antibody is responsible for the inhibition of the growth of mammalian tumors on the ground that the antibodies seem to act against the filtrate, not against the cells. This point in our opinion requires closer scrutiny, for the inhibitor has but a doubtful effect on the chicken tumor cells and yet does act on mouse tumor cells. We know from the present findings that one of the tumors used in Andrewes' experiments yields an inhibitor for mouse tumors as well as for chicken tumors, and it seems not inconceivable that the sera of fowls bearing the tumor would also contain the inhibiting factor.

It may be suggested tentatively that the property of extracts from certain relatively slow-growing strains of Chicken Tumor I, and Chicken Tumor X, by virtue of which the chicken tumor agents are neutralized and the growth of mouse sarcoma cells is inhibited, represents a definite factor distinct from the usual type of antibody.

SUMMARY

Water extracts of desiccates of certain relatively slow-growing strains of Chicken Tumors I and X, or the exudates from such tumors, definitely inhibited the growth of a mouse sarcoma (Crocker 180), and were without effect on a mouse carcinoma (Bashford 63) or Mouse Tumor S/37, a rapidly growing sarcoma derived from the stroma of a carcinoma. Extensive control tests with extracts from rapidly growing chicken tumors, and from tissues of normal and immune chickens showed no inhibiting action. There was no demonstrable action on the mouse tumors of sera from immunized rabbits, which neutralize the chicken tumor agent, nor of the sera from chickens highly immune to the chicken tumors.

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