PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR

I. The Specific Fixation by Tissues of Susceptible Animals*

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The discovery that a group of malignant tumors of the fowl could be transmitted by a cell-free extract or a desiccate of the tumor has lead to a great diversity of opinion, not only as to the classification of the tumor group, but also as to the nature of the agents possessing the tumor producing property. By many these neoplasms are classed among the virus diseases. However there are certain biological properties of these tumors and some experimental data which are difficult to reconcile with the theory that the agents are similar to those causing the virus type of infectious disease process.

Various attempts have been made to devise means of distinguishing between the filter-passing virus group, presumably living organisms, and the active substances, products of living cells, the enzyme-like group of agents. But as far as the chicken tumors are concerned, it cannot be considered that any of the earlier studies offered very definite information as to the character of the causative agents.

Certain biological agents such as bacteriophage, enzymes, toxins and antibodies, all more or less selective in the cells or substances acted upon, are first adsorbed or fixed, and sometimes apparently inactivated by the specific substratum, while non-specific cells or substances are without effect on the agents. For example, tetanus toxin, which has a selective action on the nervous system, is neutralized *in vitro* by nervous tissue from susceptible animals, while kidney, spleen and other organs from the same animals have little or no effect (1). Furthermore, the brain tissue of animals non-susceptible to tetanus toxin has practically no neutralizing effect *in vitro* on the poison (2) (3).

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There are some indications that the chicken tumor agent may be bound by tissues under certain conditions. Pentimalli (4) observed that the activity of a chicken tumor filtrate was reduced by contact with chick embryo pulp, and also by the repair tissue from a healing wound in chickens, but in a lesser degree. Leucocytes had no activity in this respect. Deelman reports a similar observation (5).

The present work has to do with a systematic study of the action of tissues of susceptible fowls on the chicken tumor agent as compared with tissues of non-susceptible animals. A preliminary note on the subject has already been published (6).

Experimenal Method

The tumor filtrates were prepared by grinding about 5 gms. of tumor tissue with sand and then adding 100 cc. of Ringer's solution. After a thorough shaking the suspension was centrifuged to remove the sand and larger particles, and the supernatant fluid passed through a Berkefeld V filter. The tissues to be tested were ground to a fine pulp in a mortar with the addition of Ringer's solution in the ratio of about 2 gms. of tissue to 1 cc. of fluid. A measured amount of the resulting pulp was placed in a centrifuge tube and a measured amount of the Berkefeld filtrate of the tumor was added and mixed thoroughly. After a period of contact, either at room temperature or in the incubator, the mixture was centrifuged and the activity of the supernatant fluid was tested by intradermal injections in normal chickens. The activity of each filtrate was tested by the injection of an untreated sample in a similar manner.

Preliminary Experiment:—Five preliminary experiments were undertaken on a small number of animals in order to gain some idea as to the quantitative relationships and the degree of specificity of the reaction. As the technique was almost identical in the several experiments, the results are presented in tabulated form in Table I.

From these preliminary tests it would seem that the muscle of susceptible chickens definitely reduces the amount of activity of the tumor agent in the filtrate. On the other hand, the brain and liver from the same chickens, and muscle, brain and liver from rabbits have no detectable effect on the potency of the tumor agent.

Fixation and Inactivation Experiments with Muscle from Chicken, Rabbit and Pigeon:—The next step was to duplicate the above experiments on an animal more closely related to the chicken, namely the pigeon, using larger amounts of tumor filtrate.

Eight cc. of the muscle pulps were mixed with 4 cc. of fresh

TABLE I

	Mixtures	Time of contact	Resultant tumor from supernat. fluid
Exp. 1.	9 cc. chicken muscle pulp + 1 cc. tumor filtrate	3 hrs. at 31°	+
	9 cc. rabbit " " + 1 cc. " "		++++
Exp. 2.	8 cc. chicken " " + 3 cc. " "	1 hr. at 37°	++
	8 cc. rabbit " " + 3 cc. " "		++++
	3 cc. chicken brain " + 1 cc. " "		++++
	3 cc. rabbit " " + 1 cc. " "		++++
Exp. 3	8 cc. chicken muscle " + 2 cc. " "	3 hrs. at 37°	−
	8 cc. rabbit " " + 2 cc. " "		++++
	8 cc. chicken liver " + 2 cc. " "	—	++++
	8 cc. rabbit " " + 2 cc. " "	1 hr. at room temp.	++++
	3 cc. chicken brain " + 0.8 cc. " "		++++
	3 cc. rabbit " " + 0.8 cc. " "		++++
Exp. 4	8 cc. chicken muscle " + 2 cc. " "	3 hrs. at 37°	−
	8 cc. rabbit " " + 2 cc. " "	1 hr. at room temp.	++
Exp. 5	8 cc. chicken " " + 2 cc. " "	5 hours	−
	8 cc. rabbit " " + 2 cc. " "		++++

tumor filtrate and allowed to remain in contact at room temperature for 3 hours for Experiment 12 and 14, and 4 to 6 hours for all the others. One cc. of the supernatant fluids was injected intracutaneously, and a similar amount of the tissue pulp in the breast muscle of normal chickens. The results are given in Table II.

TABLE II

Exp. no.	Tumors from supernatant fluids 1 cc.		Tumors from pulps 1 cc.		Tumors from filtrate alone	
	Chicken muscle	Rabbit muscle	Chicken muscle	Rabbit muscle		
6	− −	++++ ++++	−	++++	0.5 cc. ++++	1 cc. ++++
7	++ +++ ++	++ ++++ +++	+++ +++ +	+ +++ ++	0.5 cc. ++ 5 cc. ++++	1 cc. +++ 10 cc. ++++
		Pigeon muscle		Pigeon muscle		
8	−	−	−	+++	1 cc. +++	2 cc. ++++
9	− + −	− +++ −	− −	− −	0.5 cc. −	1 cc. −
10	++ +	+++ ++++	+++ +++	+++ ++++	0.5 cc. +++ 2 cc. +++	1 cc. +++ 4 cc. ++++
11	− +	++++ ++++	− +++	− +++	1 cc. +++ 5 cc. ++++	2 cc. ++++ 5 cc. ++++
12	++ −	+++ −	++++ −	+++ −	1 cc. ++++ 2 cc. −	0.5 cc. +++ 4 cc. −
13	+++ ++++ ++++	+++ +++ ++++	++ ++++	+++ ++	0.5 cc. ++++	1 cc. ++++
14	++ ++++ ++++	++ +++ ++++	++++ ++	++++ ++	1 cc. ++	5 cc. +++

It will be noted that more tumor filtrate was used with the tissue pulp in this group of experiments than in the first group. Out of the nine experiments five gave evidence of reduction in activity of the filtrate after contact with chicken muscle pulp, while one was negative, and three showed no evidence of reduction in activity of the filtrate by chicken muscle contact. With the exception of No. 6, and probably No. 8, the injection of the pulps, both from the chicken muscle and the controls, showed them to be of about equal potency in the production of tumors.

The fact that a proportion of these experiments failed to show any marked reduction in the activity of the filtrate in contact with muscle pulp from susceptible animals suggested the possibility that too active a filtrate had been used. To test this several dilutions of the filtrate were utilized in the next group of experiments. For the results see Table III.

TABLE III

Exp. no.	Tumors from supernatant fluids 1 cc.			Tumors from pulps 1 cc.	
	Chicken muscle	Rabbit muscle		Chicken muscle	Rabbit muscle
15	++ ++	+++ +++	Pure Filtrate	++++ ++	++++ +++
	+ +	+++ ++	1:1 Filtrate	++ +++	++++ ++++
	− ?	++ ?	1:2 Filtrate	+++ ++	++++ ++++
	Chicken muscle	Pigeon muscle		Chicken muscle	Pigeon muscle
16	− −	++ ++++	Pure Filtrate	?	?
	+ −	++ +++	1:1 Filtrate	++++	++++
	+ −	+++ +++	1:2 Filtrate	++	+++
	+ +	+++ ++	1:3 Filtrate	+	+++

In addition to the animals included in Table III, each of the two sets of supernatant fluids of experiments 15 and 16 from the three dilutions of filtrate were tested on the same chicken, 0.2 cc. of each being injected intradermally. The results are summarized in Table IV.

TABLE IV

Tumors from Supernatant Fluids

Exp. no.		Chicken muscle	Rabbit muscle
15	Pure Filtrate	++	+++
	1:1 Filtrate	++	++
	1:2 Filtrate	+	+++
16	Pure Filtrate	(4) −	(8) ++++
	1:1 Filtrate	(3) −	(7) +++
	1:2 Filtrate	(2) −	(6) ++
	1:3 Filtrate	(1) +	(5) ++

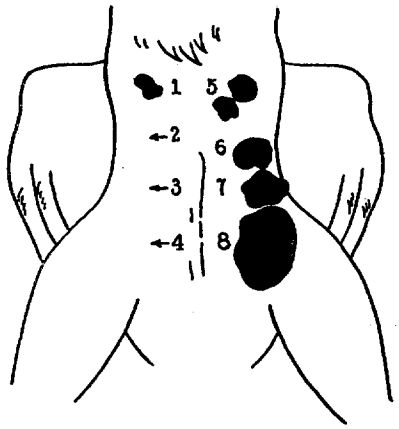

FIG. 1

Fig. 1 gives a schematic representation of the chicken used in Experiment 16, showing the relative sizes of the tumors 16 days after intradermal inocculation.

It appears from these experiments that the amount of tumor agent fixed or inactivated by chicken muscle has a definite quantitative limit.

The difference in activity between the muscle of susceptible animals and of resistant ones is more clearly demonstrated when the filtrates are diluted. The results of the injection of the muscle pulps would suggest that the fixation power of the chicken muscle is stronger than its inactivating power.

Fixation and Inactivation by Desiccated Muscle:—It was desirable to know whether the properties found in the chicken muscle were properties of the living cell in the ground tissue or were reactions between the tumor agent and the tissue constituents. The following experiment shows that dried chicken muscle is able to fix and inactivate the chicken tumor agent almost if not quite as actively as the fresh pulp.

Experiment 17:—The dry muscle used in this experiment was from the same fowl as that used in the fresh state in Experiment 6, when complete inactivation of the filtrate had taken place. The tissues used were dried in a vacuum over sulphuric acid, and then ground to a coarse powder. One gram of each muscle desiccate was mixed with 2.5 gms. of Ringer's solution and after the addition of 2 cc. of fresh tumor filtrate they were kept for two hours at 36°C. and three hours at room temperature. After centrifuging the mixture 1 cc. of each was injected intradermally in normal chickens, and the pulps were given in the same amounts intramuscularly. The activity of the filtrate alone was tested by the injection of 5 cc. intramuscularly. The results are given in Table V.

TABLE V

Tumors from supernatant fluid 1 cc.		Tumors from pulps 1 cc.		Tumors of control from 5 cc. of filtrate alone	
Chicken muscle	Rabbit muscle	Chicken muscle	Rabbit muscle		
+	++++	+	++++	++++	++++

The experiment shows quite clearly the inactivation as well as the fixation of the agent by the dry chicken muscle.

Fixation and Inactivation Experiments with Brain and Liver from Chicken, Rabbit and Pigeon:—The same relative proportions of the organ pulps and fresh tumor filtrate were used as those used in the foregoing experiments with muscles. As indicated by the experiment number, these tests were performed simultaneously with the muscle experiments, the length of contact being the same. The results are given in Tables VI and VII.

TABLE VI

Brain

Exp. no.	Tumors from supernatant fluid 1 cc.		Tumors from pulps 1 cc.		Control tumors from filtrate alone	
	Chicken brain	Rabbit brain	Chicken brain	Rabbit brain	Right side	Left side
7	++ ++	++ ++	++ ++	++ ++	0.5 cc. ++	1 cc. +++
18	+++ ++	+++ ++	+++	+++	.5 cc. –	10 cc. –
		Pigeon brain		Pigeon brain		
10	+++	+++	++++ ++++	++++ ++++	0.5 cc. +++ 2 cc. +++	1 cc. +++ 4 cc. ++++
12	– –	– –	+++	+++	1 cc. ++++ 2 cc. –	0.5 cc. +++ 4 cc. –

TABLE VII

Liver

Exp. no.	Tumors from supernatant fluids 1 cc.		Tumors from pulps 1 cc.		Control tumors from filtrate alone	
	Chicken liver	Rabbit liver	Chicken liver	Rabbit liver	Right side	Left side
7	++ + ++	++ + ++	+++ +++ ++	+++ +++ +	0.5 cc. ++ 5 cc. ++++	1 cc. +++ 10 cc. ++++
18	++ +++ ++	++ +++ ++	– –	– –	0.5 cc. – 5 cc. –	1 cc. – 10 cc. –
		Pigeon liver		Pigeon liver		
13	+++ +++ +++	+ +++ +++	++++ ++++	++ ++	0.5 cc. ++++	1 cc. ++++
14	++++ ++ +	++++ ++++ +	+++ +++	+++ ++	0.5 cc. ++	1 cc. ++++

It will be noticed in Experiments 7, 13 and 14 that the simultaneous experiments dealing with muscle gave negative results, due to the excessive activity of the filtrate. Therefore, as far as chicken liver and brain are concerned, these experiments show at least that they are not endowed with a stronger inactivating power than muscle. Experiments 10 and 12 show pronounced fixation with muscle so that a proper control exists for these two experiments, and as the filtrate used in Experiment 19 was weak it is possible that any fixation would have been detected in this experiment.

It seems, therefore, that not only do the liver and brain of rabbits and pigeons fail to show any fixating or inactivating properties for the chicken tumor agent, but that is true as well of the same organs of the chicken.

In addition to the experiments above, two other tissue were tested for the fixating power on the chicken tumor agent, namely, a mouse sarcoma and a non-filterable chicken tumor. In neither tissue was there any indication of fixation while two of the four control tests with chicken muscle showed complete fixation of the agent.

Attempts to Release the Agent from the Inactive Muscle-Filtrate Mixtures:—As noted by Marie and Tiffeneau (7), desiccation of the inactive mixture of brain and tetanus toxin releases the toxin. The technique was used in an attempt to release the tumor agent from the susceptible muscle.

Experiment 19:—The pulps from Experiment 11 were desiccated in a vacuum over sulphuric acid and four days later injected in an amount equivalent to 4 cc. of the fresh pulp.

The results obtained 15 and 30 days after the injection are given in Table VIII.

TABLE VIII

	Tumor from chicken musc.	Tumor from pigeon musc.		Tumor from chicken musc.	Tumor from pigeon musc.
15 days	–	+++	30 days	++	++++

It seems that the agent already fixed and inactivated is not released by desiccation. In fact, in this particular experiment, the injection

of dried pulps showed a greater contrast between the actions of the two muscle mixtures than did the fresh mixture as shown in Experiment 11.

General Comparison of Results with Muscle from Susceptible and Non-susceptible Animals:—If all the tests with muscle of susceptible chickens used in the above experiments be compared with the results with pigeon and rabbit muscle, striking contrast between the groups will be noted. (Table IX.) In the 47 tests with chicken muscle 76% showed unmistakable evidence of some fixation of the tumor agent, and in 34% this was complete. On the other hand, with the 43 tests with muscle from non-susceptible animals there were no instances of fixation. The figures for inactivation, while not based on so many tests show a strong contrast between chicken muscle on the one hand and the rabbit and pigeon muscle on the other.

TABLE IX

Results of Injection of Supernatant Fluids

Muscle from	Number chickens inoculated	Total fixation	Partial fixation	No fixation	Percentage tot. and part fixation	Percentage total fixation
Rabbit	19	0	0	19	0.0	0.0
Pigeon	24	0	0	24	0.0	0.0
Chicken	47	16	20	11	76.7	34.4

Results of Injection of Pulps

Muscle from	Number chickens inoculated	Total inactiv.	Partial inactiv.	No inactiv.	Percentage tot. and part inactiv.	Percentage total inactivation
Rabbit	12	0	1	11	8.3	0.0
Pigeon	12	0	1	11	8.3	0.0
Chicken	24	2	9	13	45.8	8.3

DISCUSSION

The evidence developed in this study seems to indicate that the agent of Chicken Tumor I is bound *in vitro* by muscle tissue from susceptible fowls while the muscle and the tissues from non-susceptible animals such as rabbit and pigeon are devoid of any such action. As

far as the present observation goes, even such non-mesenchymatous organs as the brain and liver of the susceptible fowl show no affinity for the tumor agent *in vitro*. It is of interest in this connection to note the early observations of Murphy and Rous (8) who showed that when tumor filtrate was injected into the chick embryo, tumors developed only in the mesodermal layers of the embryonic membranes.

The question naturally arises as to whether it is solely the muscle cell, the sarcolemma or both which are responsible for the binding action of the pulp. Although this point does not modify the essential nature of the phenomenon, the supposition that a transformation of the differentiated muscle cell under the action of the agent into a malignant cell does not seem theoretically unreasonable.

The interaction between the muscle of susceptible fowls and the tumor agent resembles in its specificity the binding of the antibodies by antigens, bacteriophage by the sensitive bacteria and the enzyme by the specific substratum. The muscle tumor agent combination seems to be rather stronger than some of the examples quoted, as desiccation does not release the activity. The delicacy of the tumor agent prevents more extensive attempts to dissociate the combination with muscles. It is true that certain viruses also have a high degree of specificity in animals and plants and share with the tumor agent the property of requiring living matter for their multiplication. However, it has been demonstrated (9) that at least one typical virus, the vaccine virus, is not bound or inactivated by contact with sensitive tissue from susceptible animals. This is possibly a fundamental difference between the behavior of the chicken tumor agent and the filterable agents of the virus group.

CONCLUSIONS

Ground muscle from susceptible chickens fixes in vitro in a proportion of instances the agent of the filterable Chicken Tumor I, and in a lesser degree inactivates it, whereas the muscle from resistant animals such as rabbit and pigeon, is without effect. It is shown that the power of fixation of the chicken muscle is far greater than its inactivating properties.

Brain and liver from chicken, rabbit and pigeon seem devoid of any action on the agent.

The desiccated chicken muscle tissue shares the properties of the fresh organ; and the process of desiccation does not release the agent from the inactive or slightly active mixture of fresh muscle and filtrate.

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