PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR

VII. Separation of the Associated Inhibitor from Tumor Extracts*

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(Received for publication, June 30, 1932)

The presence of an inhibitor or balancing factor associated with the causative agent in a chicken tumor was first indicated by the fact that the tumor-producing activity of the agent was greatly increased when a fraction of the tumor extract or filtrate was removed. This indication was confirmed by the demonstration that certain relatively slow-growing chicken tumors yielded the inhibitor in sufficient concentration to neutralize the tumor agent in the most active extracts (1). It has seemed desirable in the further study of the inhibitor fraction to secure it in greater concentration and freed so far as possible from the many other products contained in the crude tumor extract. The present paper is a report of the first step in this direction.

The Release of Chicken Tumor I Inhibitor after Adsorption on Aluminum Hydroxide

Previous experiments have shown that the most effective way of freeing a tumor extract of the inhibitor was to treat it with aluminum hydroxide (Willstätter Type C) (2), which under proper conditions appears to adsorb the inhibitor factor almost entirely but very little of the tumor-producing agent. The possibility of releasing the inhibitor from the aluminum hydroxide, thus freeing it from some of the impurities, was tested in the following experiments.

Experiment.—The material utilized in the experiments consisted of extracts of desiccated Chicken Tumor 1 known to yield sufficient inhibitor to neutralize active tumor filtrates, and to inhibit the growth of a mouse sarcoma (Crocker 180)

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

(4); and extracts of tumor desiccates known to be without effect on the chicken tumor or the mouse sarcoma. These materials were prepared by thoroughly extracting 1 gm. of the dry tumor powder with 30 cc. of water. After centrifuging

TABLE I

Release of Chicken Tumor Inhibitor after Adsorption on Aluminum Hydroxide

Materials inoculated	No. of inoculations	No. negative	Average size of tumors	Negative
			cm.	*per cent*
Material from slow-growing tumors				
0.2 cc. active C. T. I filtrate plus 0.2 cc. heated extract slow-growing tumor	19	18	0.6 x 0.4	94.4
0.2 cc. active C. T. I filtrate plus 0.2 cc. heated aluminum supernatant fluid of slow-growing tumor extract	19	1	2.3 x 1.8	5.3
0.2 cc. active C. T. I filtrate plus 0.2 cc. phosphate release from aluminum	19	18	1.1 x 1.0	94.4
0.2 cc. active C. T. I filtrate plus 0.2 cc. water	36	0	2.3 x 1.9	0
0.2 cc. active C. T. I filtrate plus 0.2 cc. M/15 basic sodium phosphate	14	0	2.2 x 1.9	0
Material from rapidly growing tumors				
0.2 cc. active C. T. I filtrate plus 0.2 cc. heated extract rapidly growing tumor	9	0	1.7 x 1.4	0
0.2 cc. active C. T. I filtrate plus 0.2 cc. heated aluminum supernatant fluid of rapidly growing tumor extract	9	0	2.1 x 1.6	0
0.2 cc. active C. T. I filtrate plus 0.2 cc. phosphate release from aluminum	9	0	1.9 x 1.6	0
0.2 cc. active C. T. I filtrate plus 0.2 cc. water	18	0	2.4 x 1.8	0

out the larger particles the supernatant fluid was filtered through filter paper, adjusted to about pH 7.2 and 20 cc. of this was shaken with an equal volume of Willstätter Type C aluminum hydroxide. The aluminum was then separated out

by centrifugation and washed with 10 cc. of distilled water 2 to 3 times. The wash waters were then discarded and the aluminum deposit was shaken up with 10 cc. of M/15 basic sodium phosphate at pH 9, the released material having a final pH of 8. The aluminum was removed and discarded. The eluate was heated at 52° for 30 minutes to inactivate any tumor agent which might have come through.

The tests consisted of the injection of mixtures in equal amounts of the eluate and active tumor filtrate, controlled by the injection of the active filtrate diluted with the heated aluminum supernatant fluid, with water and with M/15 basic sodium phosphate buffer. The inoculations were made with 0.4 cc. of the mixtures intradermally, so that each chicken received the test material and those of the controls as well. In the first group of experiments the extracts were prepared from tumor desiccates known to yield the inhibitor factor and in the second group from desiccates of rapidly growing tumors, known to yield no inhibiting substance. The results of 11 experiments with 152 inoculations are given in Table I.

It is evident from Table I that the inhibiting factor may be adsorbed on aluminum hydroxide and released with basic sodium phosphate. While the experiments were not designed to test quantitative differences between the original extract and the released material, yet they indicate that there is certainly no very great loss in the inhibiting property after adsorption and release. The failure of the heated extracts from the rapidly growing tumor to influence materially the tumor production by active filtrates is in line with previous observations and, as might be expected, there is no evidence of inhibiting action by the released material. That the phosphate in the released fluid is not responsible for the inhibiting action is shown not only by the controls, in which the phosphate was added to the active filtrate, but also by the tests with the released material from the rapidly growing tumor extract.

Release of Inhibitor from Chicken Tumor X after Adsorption on Aluminum Hydroxide

Chicken Tumor X, a very slow-growing fibrosarcoma, has proved a source of an inhibitor for Chicken Tumor I and Mouse Tumor 180 (3, 4). The inhibitor, judged by previous tests, is less potent than that from Chicken Tumor I, which might be due to the fact that the agent with which it is associated is less vigorous.

Experiment.—The technical procedure used in these experiments was the same as that described above. The source of the fluids tested for inhibiting action was extracts of desiccates prepared from Chicken Tumor X, which had required 9 months to a year to reach a large size. Beside the heated full extract, the supernatant fluid after the extract had been adsorbed out with aluminum hydroxide and the material released from the aluminum by treatment with basic sodium phosphate were tested against an active extract of Chicken Tumor I. In each instance the fluids to be tested were heated at 52° for 30 minutes. The controls included the active extract diluted with salt solution and with basic sodium phos-

TABLE II

Release of the Inhibitor from Chicken Tumor X after Adsorption of Aluminum Hydroxide

(Based on 8 experiments)

Materials inoculated	No. of inoculations	No. negative	Average size of tumors	Negative
			cm.	*per cent*
0.2 cc. active C. T. I filtrate plus 0.2 cc. heated extract C. T. X	21	18	0.9 x 0.8	85.7
0.2 cc. active C. T. I filtrate plus 0.2 cc. heated aluminum supernatant fluid of C. T. X	13	0	1.9 x 1.6	0
0.2 cc. active C. T. I filtrate plus 0.2 cc. phosphate release from aluminum	16	14	0.4 x 0.4	87.5
0.2 cc. active C. T. I filtrate plus 0.2 cc. M/15 basic sodium phosphate	10	0	1.8 x 1.5	0
0.2 cc. active C. T. I filtrate plus 0.2 cc. salt solution	22	0	2.2 x 1.5	0

phate. The results of the intradermal inoculations, in which each chicken received the control injections and the test inoculations as well, are given in Table II.

The results show conclusively that Chicken Tumor X contains an inhibiting factor capable of neutralizing the agent of Chicken Tumor I, and that the inhibitor is adsorbed by aluminum hydroxide and can be released from this combination. There is no indication of loss in potency of the inhibitor by this manipulation, a finding similar to that noted in the first group of experiments.

DISCUSSION

There is no doubt that it is possible to adsorb out practically all of the tumor agent from a filtrate with aluminum hydroxide when a large amount of the colloid is used (5). With the proportions used in these and the earlier experiments reported comparatively little of the agent is taken up, but the inhibiting factor seems to be entirely removed and to be recoverable from the aluminum. The amount of inhibitor lost by this treatment has not yet been accurately determined, but certainly effective quantities are present in the eluate.

SUMMARY

The inhibiting factor present in certain relatively slow-growing strains of Chicken Tumor I and in Chicken Tumor X is adsorbed from extracts of the desiccated tumors by aluminum hydroxide (Willstätter Type C) and can be released in effective quantities from this combination by treatment with basic sodium phosphate.

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