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Action of Enzymes on Causative Agent of the Chicken Sarcoma.

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Our previous experiments[1] with the non-cellular filtrate of the Rous chicken sarcoma No. 1 suggest that the causative agent of the chicken sarcoma is a complex protein-like substance (globulin), or is associated with the globulin fraction. The present investigation was undertaken to secure further information concerning the true nature of the causative agent of the chicken sarcoma. As a first step, we determined whether the causative agent was inactivated by certain proteolytic enzymes.

Trypsin (Fairchild Bros. and Foster) and pepsin (U. S. P., 1-3000) were selected for the proteolytic enzymes and their protease actions tested upon the causative agent in tumor fragments and cell-free filtrates of the Rous chicken sarcoma at the hydrogen ion concentrations, pH 8.2 for trypsin and pH 3.8 for pepsin. The pepsin shows optimum action at pH 1.5. However, this optimum pH was not employed in the present investigation since our previous studies[1] with the Rous chicken sarcoma indicated that the causative agent of the R. C. S. did not survive in a fluid adjusted to this pH.

Five or more small pieces of tumor tissue, each weighing about 5 mg., were placed in 25 cc. portions of Locke-Ringer solution previously adjusted colorimetrically to the required pH values with NaOH and HCl, and kept at 4-5°C., and to which had been added 0.125 gm. of powdered trypsin or pepsin. The flasks containing the solutions and tumor fragments were allowed to remain for 24 hours in the refrigerator at a temperature of 4-5° C. The tumor fragments were then transplanted into chicks in the usual way. Each set of experiments included the inoculation of control animals with the tumor fragments which had been immersed in a Locke-Ringer solution at pH 8.2 or 3.8 for 24 hours at 4-5° C.

The results indicate that the transplantability of the R. C. S. is markedly affected by the action of trypsin and less markedly by pepsin. Of the 13 trypsin-treated grafts, only 2 developed slowly growing tumors, while of the 13 pepsin-treated grafts, 6 developed

[1] Sugiura, K., and Benedict, S. R., *J. Cancer. Res.*, 1927, **11**, 164.

slowly growing tumors. In 16 control grafts, only one failed to develop a rapidly growing tumor.

Gross examination of the tumor tissues immersed in a trypsin or pepsin solution for 24 hours at 4-5° C. showed the tissue to be slightly softened but unchanged in shape. The microscopic examination of these enzyme treated tumor tissues showed marked degenerative changes. Most of the tumor cells were distinctly smaller and looser. The intercellular substances were digested and giant tumor cells were absent. While most of the nuclei stained normally, there remained some cells which appeared microscopically to be normal viable tumor cells. The treated tissue is entirely devoid of giant cells, but still retains traces of viable fibrous elements.

The temperature which we selected for the present study was far from the optimum temperature for enzyme action. Therefore, as a control we determined the residual enzyme effect by injecting fresh tumor tissue together with a small amount of the enzyme solution. The results of this experiment showed that the growth capacity of the fragments of R. C. S. was not reduced by a single intratumoral injection of trypsin or pepsin solution.

When the fragments of tumor tissue are immersed in the trypsin or pepsin solution, a certain amount of the enzyme is probably absorbed in the tissue. Therefore, we attempted to remove the absorbed enzymes by washing the tumor fragments several times with fresh Locke-Ringer solution. The results showed that the number of takes and failure of the tumor grafts previously treated with trypsin and pepsin and subsequently subjected to washings are practically the same as that obtained from enzyme-treated unwashed tumor grafts.

The preceding study was extended to the cell-free filtrates of the Rous chicken sarcoma No. 1. Three aqueous extracts of fresh chicken sarcoma were prepared with 2.7, 5.2 and 10.5% of aqueous suspensions and freed from the tumor cells as before.[1] Portions of the filtrate were adjusted to the required pH values. To 25 cc. portions of the adjusted fluid 0.125 gm. of powdered trypsin or pepsin were added, shaken thoroughly, and kept 24 hours at 4-5° C. At the end of this period, from 1.0 to 2.0 cc. portions of the treated and untreated fluids were separately injected into 56 normal chicks, the treated fluid into the right and untreated control fluid into the left pectoral muscles. The results showed that none of the 28 chicks inoculated with the trypsin or pepsin-treated tumor fluid developed tumor. On the other hand, 26 of the 28 chicks inoculated

with the untreated fluid developed rapidly growing sarcoma; the other 2 did not show tumor growth.

We next determined whether the heat-inactivated trypsin and pepsin will alter the capacity of the causative agent to excite tumor proliferation. In order to destroy the proteolytic energy, 10% solutions of trypsin and pepsin were subjected to a temperature of 80° C. for 15 minutes. To each 25 cc. of the adjusted original tumor fluid was added the inactivated trypsin or pepsin solution to an amount of 4.0 cc. Then the viability of the treated fluids was tested in the usual way. The injected chicks developed normally growing identical sarcomas.

During the course of the present investigation we also studied the effects of a castor bean lipase, a soy bean urease, and an amylase (Takadiastase) upon the causative agent in the Rous chicken sarcoma filtrate in a manner essentially similar to that used with the trypsin and pepsin experiments at the respective optimum pH values. The results showed that the lipolytic, deamidizing and amylolytic enzymes appeared to have no inhibiting effect on the causative agent since the majority of the injected chicks grew identical sarcomas.

Summary. 1. The causative agent of the Rous chicken sarcoma in the tumor fragments or in the cell-free tumor filtrate was inactivated by trypsin and pepsin, but was unaffected by the lipase, urease, or amylase preparations which were used as regards its capacity to produce tumor growth. 2. The causative agent was not destroyed if the proteolytic energy of trypsin and pepsin was abolished by heat inactivation. 3. Specificity of the proteolytic enzymes upon the causative agent of the chicken sarcoma suggests that the active agent is a protein or *associated in some way with protein material.*