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PRODUCTION OF INTERFERON-LIKE INHIBITOR INDUCED BY EXTRACT FROM ROUS SARCOMA

I. INHIBITION OF ROUS SARCOMA VIRUS INFECTION AND PROPERTIES OF THE INHIBITOR PRDUCED[1]

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SUMMARY Chick embryo cells treated with RNA preparation from Rous sarcoma tissues had reduced susceptibility to infection by Rous sarcoma virus (RSV), Bryan's high titer strain. The same RNA preparation induced chick embryo cells to produce an inhibitor of growth of RSV in the culture medium. This inducing substance was resistant to ribonuclease (RNase) and deoxyribonuclease (DNase). The inhibitor produced was shown to be non-dialyzable, and to be destroyed by trypsin or bacterial proteinase. Dialysis against solution of pH 2 or heat treatment at 56°C for 20 min had no effect on the activity of the inhibitor. From its properties, this inhibitor seems to be an interferon. The chemical nature of the tumor RNA preparation, which induced chick embryo cells to produce the interferon, was discussed.

INTRODUCTION

An interferon-like inhibitor was shown to be produced by chick embryo cells exposed to RSV (BADER, 1962). On the other hand, ISAACS (1963) suggested that production of interferon might represent a response of cells to the presence of any foreign nucleic acid, and he demonstrated that RNA of homologous cells treated with nitrous acid induced interferon production.

On examining the infectivity of RNA prepared from Rous sarcoma tissues, the author found that chick embryo cells treated with this RNA preparation had rather low susceptibility to RSV, and that an interferon-like inhibitor was produced in the culture fluids of chick embryo cells treated with this RNA preparation.

This paper reports the production of an interferon-like inhibitor by chick embryo cells treated with an RNA preparation extracted from Rous sarcoma tissues by the phenol method.

1 An abstract of this report was presented at the Japanese Annual Meeting of Bacteriology, Tokyo, 1965.

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MATERIALS AND METHODS

The RSV used in these experiments was Bryan's high titer strain. Tumor tissues which were formed on the chorioallantoic menbranes (CAM) of chick embryos infected with RSV were used for preparation of virus suspension. Virus was titrated as pock forming units (PFU) on CAM (DOUGHERTY *et al.*, 1960). Primary cultures of chick embryo cells were prepared from 10 day old embryos by trypsin treatment, and 25×10^8 cells were plated per 120 cm^2 of Roux bottle. Growth medium was a mixture of Eagle's basal medium, 10% tryptose phosphate broth (Difco), and 10% calf serum. The gas phase was 5% CO_2 in air. Free cells prepared by trypsin treatment of the primary culture after 2–3 days incubation, were cultured at a concentration of 5×10^7 cells per 40 cm^2 of culture bottle. Monolayer cell cultures were obtained after overnight incubation, and used for treatment with virus or inhibitor.

RNA was prepared by the cold phenol method (SANDERS, 1960) modified as follows. Tumor tissues formed on CAM infected with RSV were homogenized with 0.5% sodium lauryl sulphate (Duponol) (GROS *et al.*, 1961) in 0.02 M potassium phosphate buffer, pH 7.0, at 4°C, and an equal volume of water-saturated phenol was added. This mixture was stirred for 30–60 min at 4°C and then centrifuged. The aqueous phase was washed with water-saturated phenol and then shaken with ether. To the aqueous phase were added NaCl to 0.14 M and then 2 volumes of 95% ethanol and the mixture was allowed to stand overnight at 4°C. The precipitate thus formed was washed twice with a mixture of 95% ethanol and 0.14 M NaCl (2:1) and then dissolved in 0.02 M potassium phosphate buffer, pH 6.8, and dialyzed in Visking tubing against Earle's saline for 24 hr. The extract was centrifuged in an ultracentrifuge (Spinco Model L or " Hitachi ") at 40,000 rpm for 3 hr to remove particles. The RNA content of the supernatant was determined by the absorption at 260 mμ and by the orcinol reaction.

RESULTS AND DISCUSSION

1. *Inhibition of growth of RSV in chick embryo cell cultures treated with tumor RNA preparation*

The chick embryo cell suspension at a concentration of 3×10^7 cells/ml of Earle's saline prepared from primary cultures, was incubated with RNA preparation from tumor tissues at a RNA concentration of 100–250 μg/ml at 37°C. Two hours later, the medium was replaced by growth medium and cells were cultured for 2 days. The cells were then treated with trypsin and subcultured. The monolayer cell cultures formed after overnight incubation, were challenged with RSV at a multiplicity of infection of 0.1. After 40–60 min absorption, the monolayers were washed and cultured in growth medium. The culture fluids were titrated for RSV 2 days post infection. As shown in Table 1, the growth rate of RSV in cultures of cells treated with tumor RNA preparation was reduced to about 50% or less of that of cultures of untreated cells. When the RNase (Bovine Pancreas RNase, 5×cryst.) was added at a concentration of 50 μg/ml at the time of treatment of cells with the RNA preparation, the growth rate of the virus was restored. On the other hand,

TABLE 1 *Effect of Treatment of Chick Embryo Cells with Tumor RNA Preparation on Growth of RSV*

Exp. No.	Treatment						
	None (Virus control)	RNA	RNA + RNase	RNase	RNA + DNase	DNase	RNA from normal tissues
I.	263[a]	111	279				
II.	226	116					224
III.	129	36	150	182	23	106	

a PFU/ml.

DNase (1×cryst., Worthington Biochem. Co.) had no effect on the activity of the RNA preparation at a concentration of 70 μg/ml, and RNA prepared from the CAM of normal chick embryos as a control had no inhibitory effect on the growth of RSV. The alteration of cells by RSV was also examined by counting the foci formed on the culture of cells treated with the tumor RNA preparation, and the same results were obtained.

These experiments suggest that the RNA of Rous sarcoma tissues itself induces the reduced susceptibility of chick embryo cells to RSV infection.

2. *Production of inhibitor of growth of RSV by cells treated with tumor RNA preparation*

The culture fluids of cells treated with the tumor RNA preparation as described above, were ultracentrifuged at 40,000 rpm for 2 hr, and the supernatant was assayed for inhibititor against growth of RSV as following. Monolayer secondary cultures of chick embryo cells were incubated with 0.5 ml of the supernatant culture medium per 5×10^6 cells for 2 hr, and then after washing were challenged with RSV at a multiplicity of infection of 0.1. After absorption of virus for 40–60 min, the cultures were washed again and growth medium was added. After 2 days incubation, the culture fluids was titrated for RSV. As shown in Table 2, the titer of the RSV in the medium of the treated cultures was reduced to about 50% of that in the control cultures. As a control, the supernatant medium of the untreated cell cultures was used, and the RNA preparation from normal CAM was shown to be inactive in similar experiments. RNase and DNase were both shown to have no effect on the activity of the tumor RNA preparation.

3. *Properties of the inhibitor* (Table 3)

This inhibitor was shown to be non-dialyzable through Visking tubing (within 2 days) and to be resistant to RNase (50 μg/ml) and DNase (70 μg/ml) treatment for 3 hr at 37°C. Heating at 56°C for 20 min had little or no effect on the activity of the inhibitor. Dialysis against 0.05 M KCl-HCl buffer, pH 2.0, for 24 hr had no effect on the inhibitory activity. When the inhibitor was incubated with 0.5% trypsin or 0.05% Pronase (proteinase from *Streptomyces griseus;* Kaken, Tokyo) at 37°C for 4 hr, the inhibitory activity was completely lost. These properties of the inhibitor are very likely to be those of interferon. The experiments presented here suggest that the RNA preparation from Rous sarcoma tissues has at least two components. One of these is RNA, which induces reduced susceptibility in chick embryo cells to infection with RSV, and the other is a substance, which is resistant to both

TABLE 2 *Production of Inhibitor Induced by Tumor RNA Preparation*

Exp. No.	Treatment						
	None (Virus control)	RNA	RNA + RNase	RNase	RNA + DNase	DNase	RNA from normal tissues
I.	131[c]	64					
II.[a]	186	96					
III.[b]	149	51					
IV.	396	151					307
V.	210	113	42	138	52	178	138

a Treatment of CAM with inhibitor (2 hr) and inoculation of virus of a certain titer.
b Treatment in hypertonic medium (0.7 M NaCl in Earle's saline).
c PFU/ml.

TABLE 3 *Effect of Various Treatments on Activity of Inhibitor*

Exp. No.	Treatment						
	Virus control	Inhibitor control	Acid (pH 2)	Trypsin	Pronase	RNase	DNase
I.	373[b]	250	140				
II.[a]	210	143		180	205		
III.[b]	322		77	323	168		
IV.	627	401				207	322

a Inhibitor was treated with acid and heated at 56°C for 20 minutes after incubation with or without trypsin or Pronase.
b PFU/ml.

RNase and DNase and induces the chick embryo cells to produce an interferon-like inhibitor. Recently, KLEINSCHMIDT *et al.* (1964), demonstrated that statolon, a polysaccharide of a mould, induced interferon production. It is possible that the second substance in the tumor RNA preparation in the present experiments might also be a polysaccharide present in the RNA preparation as a contaminant, or it might be a RNase-resistant RNA hybrid with RNA or DNA. RNA, which itself induces reduced susceptibility of cells to infection by RSV, could also induce the cells to produce the interferon, but its presence could not be demonstrated in the present experiments.

The nature of these substances and their origin are being studied.

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