An Interferon Produced in Response to Infection by Herpes Simplex Virus.* (29703)

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In recent years, widespread attention has been given to a family of inhibitory agents (interferons) produced by cells in response to virus infection. These interferons have been found to suppress the replication of a wide range of RNA-containing viruses(1). There have been only a few reports on their inhibitory action against DNA-containing viruses (2,3). Very little has been reported about the position of herpes simplex virus with regard to its capabilities in stimulating interferon production or its susceptibility to interferon. Yet herpes simplex infection of man has many analogies with latent and persistent virus infections *in vitro*(4), which are at least in part controlled by interferon(3,5,6,7).

The current study was inspired by observations in this laboratory(8) that an interferon-like substance plays a crucial role in the equilibrium of a persistent herpes infection in tissue culture in the absence of antibody.

The present report describes the existence of an interferon-like substance produced in chick embryos by the GCA3 strain of herpes simplex virus.

Materials and methods. Virus strains. Vaccinia virus, maintained by passage in HeLa cells, and the Melbourne strain of influenza A (MEL), maintained by passage in the allantoic cavity of 10- to 11-day-old chick embryos, were obtained from Dr. J. Lindenmann. The vaccinia virus pools were produced in HeLa cells and titered $10^{6.2}$ to $10^{6.7}$ plaque-forming units (PFU) per ml in chick embryo fibroblasts (CEF). The MEL stocks used in these experiments titered 1280 HA units per ml. Herpes simplex virus, GCA3 strain, was obtained from Dr. T. F. McNair Scott, of the Children's Hospital of Philadelphia. The herpes stocks used in these experiments were prepared in HeLa cells and varied in titer from $10^{6.5}$ to $10^{7.1}$ PFU per ml in CEF.

Cell cultures. Chick embryo cells. Chick monolayer cell cultures were prepared by trypsinization of 9- to 10-day-old chick embryos from which head and limbs had been removed. Cultures were initiated in 30 × 30 × 60 mm soft glass bottles containing 5 ml of medium at 2 × 10^6 cells per ml. Growth medium consisted of 5 ml calf serum and 10 ml of 5% lactalbumin hydrolysate per 100 ml of Earle's balanced salt solution (BSS), with 400 units penicillin and 200 μg streptomycin per ml. Maintenance medium, which was applied 2 days after initiation of cultures, was identical to the growth medium except 10 ml 2% yeast extract was substituted for the calf serum.

Results. Production of interferon. Interferon preparations were obtained by allantoic inoculation of herpes simplex virus (HSV) into 10-day-old chick embryos. Each embryo received 1.0 ml of undiluted, inactivated HSV($10^{6.0}$-$10^{7.5}$ EID_{50} per egg). The inactivation was accomplished by exposure of the virus to ultraviolet radiation (from a 15 W General Electric germicidal lamp) for 15 minutes at a distance of 5 inches from the source. The virus was inactivated in 5 ml aliquots agitated in 100 mm Petri dishes by a magnetic stirrer. The inoculated eggs were then incubated at 37°C for 72 hours, at which time the allantoic fluids were harvested, pooled, and subjected to low speed (700 × *g*) centrifugation for 20 minutes to remove chick erythrocytes, ureates, etc. The fluids were then dialyzed at pH 2.0 for 24 hours, followed by 48-hour dialysis at pH 7.4 against distilled water or Earle's BSS. The dialyzed fluids were subjected to low speed centrifugation once again to remove

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TABLE I. Herpes Interferon Activity in Chick Embryo Cells Challenged with Herpes Simplex Virus.

Interferon dilution	Exp I			Exp II		
	Avg No. plaques*	% Plaque reduction	S.D.	Avg No. plaques†	% Plaque reduction	S.D.
Control	119.0	—	7.8	204.5	—	8.6
1:4	69.3	42	11.8	120.0	41	1.6
1:8	88.0	26	1.0	147.4	28	2.9
1:24	ND	—	—	193.0	6	5.6

* Averages computed from 4 cultures per dilution. † Averages computed from 5 cultures per dilution. S.D. = Standard deviation.

precipitates. The fluids were sometimes ultracentrifuged to remove residual HSV, and/or lyophilized prior to storage at 4°C. Our investigations have shown no detectable difference in the amount of inhibition by fluids from which residual HSV had been removed by ultracentrifugation at 105,000 × *g* for 2 hours following dialysis, and those merely dialyzed at pH 2.0. Although the potency of individual lots of interferon varied, interferon activity was always present.

Interferon assay. Herpes interferon was assayed in monolayer cultures of chick embryo cells, using a plaque reduction method similar to that described by Lindenmann and Gifford(9), with HSV or vaccinia as challenge viruses. Alternatively, interferon was assayed by determining inhibition in hemagglutinin production in CEF with MEL used as the challenge virus. Monolayers of chick embryo cells received maintenance medium, interferon, and challenge virus simultaneously. Maintenance medium was prepared to give a final concentration of 1 × after addition of an appropriate quantity of interferon and Earle's BSS as diluent to a volume of 2.4 ml. Each bottle then received 0.2 ml of an appropriate virus dilution, bringing the final volume to 2.6 ml. The cultures were incubated undisturbed at 36°C.

Cultures challenged with HSV or vaccinia were incubated until plaques had reached maximum size, and prior to the appearance of secondary plaques (approximately 30 hours for HSV and 44 hours for vaccinia). Cultures were then stained with crystal violet, and plaque counts were made under a photographic enlarger at a magnification of 45 ×. Interferon activity was measured as percentage reduction in the plaque count of treated

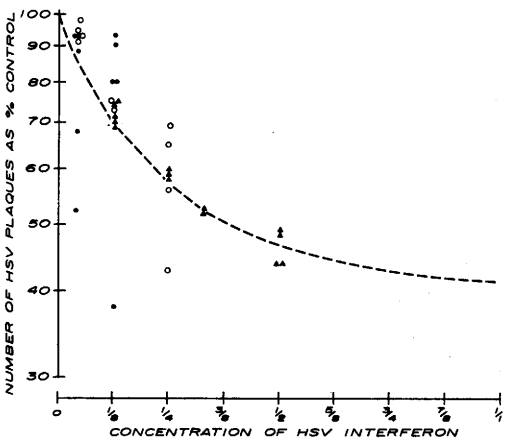

FIG. 1. Variation of inhibition of plaque formation with dose of herpes interferon. The points represent plaque counts. Three separate experiments are signified by the solid, open, and triangular points. The dotted line was drawn through an average of all determinations.

cultures as compared with controls receiving no interferon. The results obtained in representative experiments using HSV as the challenge virus are presented in Table I and Fig. 1. Sensitivities of HSV and vaccinia to herpes interferon are compared in Table II. There is apparently little difference in the sensitivities of these two viruses to herpes interferon.

When MEL was used as the challenge

TABLE II. Relative Sensitivity of Herpes and Vaccinia Viruses to Herpes Interferon as Determined by Plaque Reduction in Chick Embryo Cells.

Challenge virus	Interferon dilution	Percentage plaque reduction
Herpes simplex	1:2	54
	1:4	42
	1:8	27
Vaccinia	1:4	53
	1:8	30

TABLE III. Herpes Interferon Activity in Chick Embryo Cells Challenged with Melbourne Influenza.

Exp	Dilution of challenge virus*	Amt of HA (log_2) in challenge dose	Avg HA titer† (log_2) Controls	Treated	Inhibition of yield In log_2	In %
1	10^{-2}	1.4	4.7	4.0	.7	37
	10^{-3}	−2.0	4.0	1.3	2.7	85
2	10^{-2}	1.4	4.7	3.0	1.7	70
	10^{-3}	−2.0	2.2	.0	2.2	78

* Virus had a titer of 1280 HA units per ml.
† Avg titer of 3 to 5 samples, determined by hemagglutination test.

virus, the cultures were incubated for 72 hours, at which time the culture fluids were harvested and stored at 4°C. Interferon activity was determined by the hemagglutination test on the stored culture fluids using guinea pig erythrocytes. Virus end point was pressed as the log_2 of the highest dilution to show definite hemagglutination. Interference was expressed as the difference in log_2 between interferon-treated and control cultures (Table III).

Physical and chemical properties. The physicochemical properties of the interfering substance were shown to be very similar to those reported for other interferon-like substances, and are summarized in Table IV.

Viral specificity. Herpes interferon has been shown to be effective in inhibiting the growth of HSV, vaccinia, and MEL in chick embryo cells in culture.

Cell specificity. Herpes interferon prepared in chick embryos was tested in cultures of 2 strains of chick embryos without detectable difference in interference. Preliminary experiments suggest that herpes interferon prepared in chick embryos may be effective in some mammalian cell lines.

To determine whether the antiviral substance would be effective after adsorption of virus, an experiment was conducted in which the challenge virus was allowed to adsorb to the cell sheet at 37°C for one hour prior to addition of the interferon-containing maintenance medium. The inhibitor produced a suppressive effect similar to that obtained when inhibitor and virus were introduced simultaneously.

Discussion. Previous work has relegated herpes simplex virus to a peculiar position of inertness with regard to production of interferon and resistance to the action of interferon. Using the GCA3 strain of herpes simplex virus, we have found that an interferon-like substance is produced by the cells of the chick embryo. The substance suppresses the activities of vaccinia, herpes, and influenza viruses. Several of the chemical and physical properties of this substance make it identifiable as interferon. The fact that the GCA3 strain of herpes simplex virus could

TABLE IV. Comparison of Physical and Chemical Properties of HSV and MEL Interferons.

Property tested	HSV interferon	MEL interferon*
Sedimentability	Unsedimentable at 105,000 *g* for 4 hr	Unsedimentable at 105,000 *g* for 4 hr
Dialysis	Undialyzable	Undialyzable
pH stability	Stable pH 2.0-8.5	Stable pH 2.0-8.5
Storage at 4°C	" >6 mo	" >6 mo
80°C for 1 hr	Partial inactivation	Marked inactivation
Trypsin	Marked "	Near total "
Ether	Slight "	Slight "
Glycolysis	Markedly increased	Markedly increased
DNase (10-100 γ)	No loss	NT†
RNase (10-100 γ)	" "	NT

* Prepared in our laboratory by method of Gifford, Toy, and Lindenmann (10).
† Not tested.

be used successfully for both induction of interferon and assay of interferon action clearly shows that certain strains of this virus are both stimulatory and responsive in the interferon system.

Existence of an interferon produced by cells infected with HSV suggests that interferon may play an important role in human infections of HSV. Since herpes antibody titers do not vary significantly in humans prior to or following the recurrence of lesions (4,11), it must be assumed that some other factor is responsible for the host-virus equilibrium. Results obtained in our laboratory (8) have demonstrated that antibodies play a secondary role in the course of herpes infection *in vitro,* and that interferon exerts the major effect in determining the outcome of such infections. Thus, it is quite conceivable that interferon may play a predominant role in the classic latency of herpes in humans.

Summary. The production of an interferon-like substance by chick embryos infected with herpes simplex virus is described. The physical, chemical, and biological properties of herpes interferon are demonstrated to be similar to those of previously reported interferons. Speculation is made on the possible role of interferon in the classic example of latency in humans—infection by herpes simplex virus.

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Effects of Retrograde Injections of Vasopressin into the Upper Urinary Tract of Hydrated Rats. (29704)

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That arginine-vasotocin can act when delivered to the tubular cells from the blood side has been shown by infusing the hormone into the renal portal circulation of the chicken(3). It has also been shown that retrograde flow into the distal part of the nephron can occur when a slight hydrostatic pressure is applied(1,2,4). In the present study arginine-vasopressin was injected through the ureter in water-loaded rats to try to determine whether the hormone can act when present only on the luminal side of the distal tubules and collecting ducts.

Materials and methods. Trained male white rats weighing about 400 g were fasted overnight, then 4 ml/100 g of 12% (v/v) ethanol in water was given by stomach tube. Forty-five minutes later 5-10 mg/100 g of Inactin® (Promonta, Hamburg), a thiobarbituric acid derivative, was injected intraperitoneally. The trachea was cannulated, then 5 ml/100 g of 2% (v/v) ethanol in water was given orally. A lower median laparotomy was performed. No. 10 PE catheters were passed into both ureters approximately to a point 5 mm from the pelvis; the ureters were ligated, and the laparotomy was closed. The right catheter was connected to a mercury manometer through a side tube. A hydration of approximately 8% of body weight was maintained throughout using the 2% ethanol solution. When a good water diuresis (osmolality about 100 mOsm/kg) had been established after the operation, retrograde in-