Vol. XXXIX, No. 4 (August, 1958), was issued on 19.8.58.

THE BRITISH JOURNAL OF
EXPERIMENTAL PATHOLOGY

VOL. XXXIX OCTOBER, 1958 NO. 5

EFFECT OF INTERFERON ON THE GROWTH OF VIRUSES ON THE CHICK CHORION

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Received for publication March 27, 1958

ONE of the most striking characteristics of the interference phenomenon is that one virus may interfere with the growth of a number of unrelated viruses. Thus, Henle and Henle (1945) showed that inactivated influenza virus inhibited the growth of influenza A and B, Western equine encephalitis and epidemic kerato-conjunctivitis virus (now thought to be St. Louis encephalitis virus) in the allantoic cavity of the chick embryo. The same lack of specificity is shown by interferon, a product of the virus interference reaction, which was found to inhibit a wide range of viruses grown in pieces of chick chorio-allantoic membrane *in vitro* (Isaacs, Lindenmann and Valentine, 1957). Among the viruses inhibited by interferon in this way was vaccinia virus, and attempts were therefore made to see whether interferon would inhibit the development of pocks by vaccinia virus grown *in ovo*. First attempts were discouraging, however (Lindenmann, Burke and Isaacs, 1957), and as we had no method of following the distribution of interferon in the egg after it was inoculated on the chorion it was difficult to know at what concentration of interferon to aim. Recently, following an incidental observation, we were able to choose a dose of interferon which might be expected to produce interference and this dose caused a highly significant inhibition of the development of pocks by vaccinia virus. These findings, together with the result of similar experiments with other pock-producing viruses grown on the chick chorion, are described in this paper.

MATERIALS AND METHODS

U.V.-irradiated influenza virus.—The MEL strain of influenza virus A was grown in the allantoic cavity of 10-day chick embryos. Virus was partially purified from infected allantoic fluid by adsorption on to chick red cells at 0° followed by elution into saline at 37°. It was irradiated by exposure for 2 min. in shallow layers to a mercury germicidal lamp giving maximal emission at 2537 Å. The dose was estimated as $1{\cdot}9 \times 10^6$ ergs per sq. cm. by the

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method of Forbes and Heidt (1934). For purposes of calculation all the emission was assumed to be at 2537 Å. Actually about 75 per cent of the emission was below 3000 Å, *i.e.*, was absorbed by glacial acetic acid.

Pock-producing viruses

Vaccinia : An egg-adapted strain.
Cow-pox : Strain kindly supplied by the Virus Reference Laboratory, Colindale.
Ectromelia : Hampstead strain.
Herpes simplex : Nash strain.

All these strains were stored in capillary tubes at — 70° and used at a suitable dilution as determined by previous titration of the contents of one capillary. Embryos were inoculated at 12 days on the " dropped " portion of the chorio-allantoic membrane by a slight modification of the method of Beveridge and Burnet (1946). They were then incubated at 37° for 2 days (vaccinia, ectromelia and herpes simplex) or 3 days (cow-pox), the dropped portions removed and the pocks counted.

Interferon.—Interferon was prepared by mixing U.V.-irradiated influenza virus with whole chorio-allantoic membranes *in vitro*, washing the membranes after 2–3 hours at 37° and then incubating them in a shaking machine suspended in Earle's buffered salt solution overnight at 37° (Lindenmann *et al.*, 1957). One ml. of a 1/20 dilution of interferon prepared in this way reduced the yield of influenza virus (MEL) to about 5 per cent of that of the controls when tested in pieces of chorio-allantoic membrane *in vitro*. Interferon was concentrated from the fluid medium by precipitation with saturated ammonium sulphate and this was followed by dialysis at pH 2 overnight and then dialysis against Earle's solution (pH 7·5) overnight.

Interference experiment on the chorion.—The chorio-allantoic membranes of 12-day embryos were " dropped " as described above and they were then inoculated on the chorionic surface of the dropped portion with 0·5 ml. of interferon or buffer. After overnight incubation at 37° the eggs were inoculated on the chorion with challenge virus suitably diluted in a volume of 0·1 ml. After a further period of incubation the dropped portions of the membrane were cut out, washed and the pocks counted under low magnification. Normally 6–8 eggs were used for each group.

Vaccinial haemagglutinin titration.—In the case of vaccinia, cow-pox and ectromelia viruses interference was further assessed by measuring the amount of haemagglutinin which developed in the chorio-allantoic membranes. Membranes were pooled in groups of 2 or 3 and ground with glass powder. The homogenates were suspended in saline (1 ml. per membrane) spun and the supernatants titrated with vaccinia-positive chick red cells for haemagglutinin. The titration was carried out as for influenzal haemagglutinin except that the titrations were incubated at 37°.

Influenzal interference experiment.—The portions of chorio-allantoic membrane under investigation were cut out, washed and placed in test-tubes along with 1 ml. of buffer containing MEL diluted 10^{-3} as challenge virus. Six pieces of membrane were used in each experimental group. After incubation in the roller drum at 37° for 2 days the fluids were titrated for haemagglutinin and the geometric mean titre for each group calculated. Interference is shown in Table I as the yield of haemagglutinin compared with the yield in the controls. A yield of 25 per cent or less than that of the control is statistically significant at the 1 per cent level (Isaacs and Lindenmann, 1957).

RESULTS

It has been shown by Henle, Henle and Kirber (1947) that interference is localised to the cells actually in contact with the interfering virus ; interference by inactive influenza virus in the chick amnion had no effect on the growth of virus in the allantoic cavity and vice versa. We were surprised therefore when we observed that irradiated influenza virus inoculated on the chick chorion induced interference in the cells of the allantoic surface. This could be shown experimentally by inoculating U.V.-irradiated influenza virus on the chick chorion, incubating the eggs overnight, and cutting out the dropped portion of the membrane. This

was then tested for its ability to support the growth of influenza virus *in vitro* as compared with uninoculated controls. First experiments showed that inoculation on the chorion had induced interference in the allantoic cells and since it was known that live influenza virus inoculated on the chick chorion does not spread to the allantoic cells of the intact chorio-allantoic membrane (Fulton and Isaacs, 1953) the possibility was considered that some interfering substance might be produced in the chorion and diffuse through to the allantois. When it was found that live influenza virus similarly inoculated on the chorion did not induce interference in the allantois, it seemed likely that the hypothetical diffusing substance might be interferon, since the irradiated influenza virus was known to produce interferon after inoculation on the chick chorion whereas live virus did not (Burke and Isaacs, 1958). This possibility was greatly strengthened when it was found that interferon itself when similarly inoculated on the chorion induced interference in the allantois. An experiment illustrating these findings is shown in Table I.

TABLE I.—*Diffusion of Interferon through the Chorio-allantoic Membrane*

		Inoculum	Dose	Interference—expressed as percentage yield compared with control
Group No.	1	U.V.—MEL	2800 A.D.*	6
	2	U.V.—MEL (opposite side)	2800 A.D.	87
	3	Live MEL	2400 A.D.	95
	4	Interferon	0·5 ml. (× 10 concentrate)	3

* Agglutinating doses.

The inocula shown were inoculated on to the dropped portion of the chorion of groups of six 10-day embryos along with buffer given to control eggs. After overnight incubation at 37° the dropped portions of the membranes in groups Nos. 1, 3 and 4 and similar sized portions of the opposite side to the dropped portion in group No. 2 were cut out. The membrane pieces were washed and placed separately in test tubes with 1 ml. buffer/tube containing MEL diluted 10^{-3} as challenge virus. The interference is expressed as mean haemagglutinin yield in a group of 6 membranes as compared with corresponding controls.

Table I shows, in addition, that the interference induced by U.V.-irradiated virus was localised to the "dropped" portion of the membrane, since when corresponding pieces of chorio-allantoic membrane from the opposite side of the same egg were removed and tested along with the appropriate controls no interference could be demonstrated. This experiment showed that interferon had spread locally from the chorionic cells through to the neighbouring allantoic cells. From this it could be concluded that 0·5 ml. of a 10-fold concentrate of interferon prepared as described should cause highly significant interference in the dropped portion of the chorio-allantoic membrane, and this amount was tested for its action against vaccinia virus grown in this site.

Effect of interferon on the growth of vaccinia virus on the chick chorion

Interferon, in the same amount as was used in the above experiment, was inoculated on to the chorion of 12-day embryos, followed 24 hr. later by an inoculation of suitably diluted vaccinia virus. Control eggs were inoculated with buffer in place of interferon. Interference was assessed by pock counts and titrations of

vaccinial haemagglutinin in membrane extracts. The results of a representative experiment are shown in Table II.

TABLE II.—*Action of Interferon on the Growth of Vaccinia on the Chick Chorion*

1st inoculum	Pock counts in individual eggs	Mean and standard deviation	Vaccinial* haemagglutinin titre
Interferon	0, 7, 6, 14, 3, 0, 4, 4	5±4	1, 1
Buffer	75, 65, 76, 60, 57, 65, 30	61±15	16, 32

* Titre of extract of pools of 4 membranes.

Table II shows that there was a highly significant depression of the pock count in the interferon-treated eggs, and interference was also shown by a corresponding reduction of the vaccinial haemagglutinin yield. In this and in other experiments the ratio of the titre by pock count to the haemagglutinin titre of membranes was roughly the same in interferon-treated and control eggs.

In the interferon-treated membranes, the pocks appeared to be macroscopically normal and of the same size as those in control eggs. No other macroscopic changes were seen in interferon-treated membranes and the effect of this dose of interferon appeared to resemble that produced by diluting the vaccinia inoculum about 10-fold.

Effect of interferon on the growth of other viruses on the chick chorion

The experiment shown in Table II was repeated along with similar experiments with cow-pox, ectromelia and herpes simplex viruses. Typical results are shown in Table III.

TABLE III.—*Effect of Interferon on the Pock Count of Different Viruses Grown on the Chick Chorion*

Experiment	1st inoculum	Challenge virus	Mean pock count ± S.D.
I	Interferon	Vaccinia	14±10
	Buffer	,,	46±28
	Interferon	Cow-pox	29±16
	Buffer	,,	85±22
II	Interferon	Vaccinia	9·5±4
	Buffer	,,	104±26
	Interferon	Herpes simplex	10±8
	Buffer	,,	25±19

The depression of the cow-pox pock count was similar to that of vaccinia when the same preparation of interferon was used.

Ectromelia was unsatisfactory since the egg-to-egg variation in pock counts was extremely high, and for this reason the pock counts are too unreliable to include in Table III. However some depression of pock count seems to have occurred in interferon-treated eggs and the haemagglutinin content fell to 30 per cent in an experiment where the vaccinia pock count was reduced to 30 per cent.

The interference found with herpes simplex virus was hardly significant since here again there was considerable variation in pock count from egg to egg.

DISCUSSION

U.V.-irradiated influenza virus inoculated on the " dropped " portion of the chick chorion was found to be capable of inhibiting the growth of influenza virus in the corresponding part of the allantois. The interference did not spread to the chorio-allantoic membrane on the opposite side of the same eggs, and the experiments suggest that the irradiated virus produced interferon in the chorionic cells, and that this then diffused through the membrane to the neighbouring allantoic cells. The practical usefulness of this observation was that it allowed us to find a dose of interferon which would be expected to induce interference in the dropped portion of the membrane.

This dose of interferon caused highly significant inhibition of the development of pocks and haemagglutinin by vaccinia virus. However the same dose of interferon did not inhibit significantly the development of pocks by herpes virus although this virus might be inhibited by higher doses of interferon. Nevertheless the fact that vaccinia virus was greatly inhibited at a concentration of interferon which did not significantly inhibit the development of herpes simplex virus, makes it unlikely that the inhibition of vaccinia virus is due simply to a toxic effect on the membrane which would render it incapable of supporting virus growth. The closely related cow-pox and ectromelia viruses were inhibited to about the same degree as vaccinia.

SUMMARY

Interferon inoculated on the chick chorion *in ovo* diffused through to the neighbouring allantoic cells and interfered with their ability to support the growth of influenza virus *in vitro*. The same dose of interferon greatly inhibited the development of pocks and hamagglutinin by vaccinia, but did not significantly interfere with pock development by herpes simplex virus. Cow-pox and ectromelia were inhibited to about the same extent as vaccinia virus.

We should like to thank Dr. C. H. Andrewes, F.R.S. for his advice and criticism and Miss V. Coker and Mr. V. G. Law for their able technical assistance.

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