STUDIES ON THE PRODUCTION, MODE OF ACTION AND PROPERTIES OF INTERFERON

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THE phenomenon of viral interference has been studied extensively in the past twenty years, and particular attention has been paid to the inhibitory effect on influenza virus growth produced by previous inoculation with killed virus of either the same strain or a different serological type.

In a previous paper (Isaacs and Lindenmann, 1957) we described the occurrence of a product of influenza viral interference which we called " interferon ". Interferon was produced by the interaction of heat-inactivated influenza virus and chick chorio-allantoic membranes *in vitro*, and its presence was revealed by its ability to induce interference in fresh pieces of chorio-allantoic membrane. It was also shown that interferon could be distinguished from heat-inactivated influenza virus by several properties ; thus interferon was non-haemagglutinating, its activity was not neutralized by viral antiserum, it was not sedimented by centrifugation at $100,000\,g$ and surprisingly, it was held up by filtration through gradocol membranes of average pore diameter $0\cdot5$–1 μ. However, it was not possible to decide whether interferon was a cellular product formed as a reaction to the virus, a part of the heated virus itself, or whether it represented an abortive attempt at virus multiplication. In the present paper we describe further experiments relating to the production, mode of action and properties of interferon.

MATERIALS AND METHODS

Interfering virus.—In most experiments the Melbourne strain of influenza virus A was used as freshly harvested allantoic fluid, mixed with a 2 per cent sodium citrate solution in normal saline and borate buffer pH 8·5, in the ratio 6 parts virus, 2 parts citrate-saline and 1 part borate buffer. This was heated at 56° for 1 hr., which abolishes the infectivity of the virus while retaining its interfering activity. The heated virus is referred to as heated MEL.

In a few experiments the virus was inactivated with ultra-violet light. It was first partially purified by absorption from infected allantoic fluid on to chick red cells at 0° and elution at 37° into normal saline. The eluate was then irradiated in shallow layers with a mercury vapour germicidal lamp giving maximal emission at 2537 Å. The time for irradiation was decided empirically by using double the minimal time necessary to inactivate the infectivity of the virus preparations used.

Other virus strains.—Other viruses used were the PR8 strain of influenza A, the haemagglutinating virus of Japan or Sendai virus (Kuroya, Ishida and Shiratori, 1953) and vaccinia virus. All strains were kept in capillary tubes at − 70°.

Buffers.—The buffer which we prefer is that described by Earle (see Parker, 1950), but at one point in these studies a bicarbonate buffer could not be used and a glucosol-phosphate buffer was used instead (see Fulton and Armitage, 1951).

Chorio-allantoic membranes.—Membranes from 10– or 11-day eggs were used. If whole membranes were required the shell was removed from the pointed end of the eggs, the egg

contents poured out and the membranes were then removed and washed in two changes of buffer. When small pieces of membrane were required pieces of shell with membrane attached were cut out of the de-embryonated egg, and the membrane pieces were then detached and washed. Six or seven pieces were taken from each egg and membranes were pooled from a group of eggs and randomised.

Interference and challenge

An interference experiment was usually carried out as follows : small pieces of membrane were placed in $6 \times \frac{5}{8}$ in. $(15 \times 1.5$ cm.) test tubes, 1 piece in each tube along with 1 ml. of test or control material. One hundred units of penicillin were added per ml. of fluid and six tubes were used for each experimental group. The tubes were stoppered and placed in a roller drum at 37° (8 rev./hr.). After 18–24 hr. incubation the fluids were removed and replaced with 1 ml. buffer in which MEL virus at a dilution of 10^{-3} was incorporated. In some experiments the membranes were washed before challenge with MEL but usually this step was not necessary. The tubes were then placed in the roller drum for a further 48 hr. at 37° after which the fluids were titrated individually for their haemagglutinin content. A 48 hr. challenge period gave more satisfactory results than a 24 hr. period. Where this procedure was varied it is indicated in the text.

Haemagglutinin titrations

Two-fold dilutions (0.25 ml.) of test virus were made in normal saline using automatic pipettes and plastic plates. To each dilution 0.25 ml. of a 0.5 per cent suspension of chick red blood cells was added, and the cells allowed to settle. That pattern which showed partial agglutination was taken as the end-point, and it was read by interpolation if necessary. One agglutinating dose (A.D.) is defined as the amount of virus present at the partial agglutination end-point. The readings in the tables are given as $\log_2$, i.e., tube number of a series of two-fold dilutions. Thus if the end-point of agglutination occurs at a 1/2 dilution the reading is taken as $\log_2 = 1$. If the end-point of agglutination occurs at a 1/4 dilution the reading is taken as $\log_2 = 2$. A 1/1 dilution end-point has a $\log_2 = 0$ and material which did not agglutinate red cells at a 1/1 dilution was given an arbitrary score of -1.

Assessment of results

The results are given in the tables as geometric mean haemagglutinin titres of a group of six fluids, the titres being expressed as the $\log_2$ of the dilution at the haemagglutination end-point. The tables also show the haemagglutinin yield in each group expressed as the per cent of the corresponding control. Evidence has previously been given (Isaacs and Lindenmann, 1957) that if an experimental group of six tubes showed a geometric mean titre of one $\log_2$ unit less than a group of six controls tested at the same time this indicated a slight but statistically significant degree of interference, and a difference of two $\log_2$ units indicated definite interference. Such differences correspond to a 50 per cent yield and a 25 per cent yield compared with the controls, and small arithmetic differences in the percentage yield have a much greater significance at low than at high percentage yields.

By making use of the observation that a linear relation exists between the concentration of interferon and the degree to which the virus yield is depressed (Isaacs, Lindenmann and Valentine, 1957) it was possible to make a rough comparison of the potency of different preparations.

Pressure dialysis.—Interferon was concentrated by pressure dialysis. Fluids were placed in large cellophane Visking sacs attached to a glass bulb as reservoir, and immersed in a cylinder of buffer and compressed air was forced into the bulb to a pressure of 600 mm. mercury. The sacs were left for 48 hr. at 2° when the volume had usually decreased to about 1/10th. Some insoluble material usually precipitated out but there was no significant loss of interferon activity.

Enzymes.—Trypsin : a crystalline preparation from Armour and Co. Ribonuclease was kindly supplied by our colleague Dr. R. R. Porter. Crystalline deoxyribonuclease : the Worthington Biochemical Co. Receptor-destroying enzyme of *V. cholerae* (RDE) : a crude extract was obtained from Phillips-Roxane Co. and from this the enzyme was partially purified by absorption on chick red cells at 0° and elution into saline (Burnet and Stone, 1947). As titrated by the method of Burnet and Stone eluates usually gave a titre of about 640 units/ml.

Miscellaneous.—In experiments involving precipitation of interferon with ammonium sulphate or addition of sodium periodate or urea, it was necessary to remove these reagents before interferon could be tested biologically. Small-molecular substances such as these were removed by dialysis against large volumes of buffer at 2°, usually with one or two changes of buffer at daily intervals.

RESULTS

Production of Interferon

In previous experiments interferon was produced in small amounts in test tubes. For the larger yields required for isolation and characterisation of interferon it was necessary to simplify and standardise its production. At the same time the effect of variables on the yield was studied.

Production in bottles.—Roux bottles of capacity 1 l. were found convenient. Entire chorio-allantoic membranes were removed from 10- or 11-day chick embryos, washed in buffer and 10 membranes placed in a Roux bottle with 50 ml. of suitably diluted heated MEL virus. Such bottles were then placed at 37° in a horizontal shaking machine delivering 86 strokes/min. (to-and-fro) with an amplitude of about 1 in. (2·5 cm.). After 3 hr. incubation the membranes were removed, washed twice in buffer and re-suspended in 50 ml. volumes of buffer in fresh bottles. These were returned to the shaking machine and incubated at 37° overnight. The fluid was then removed and centrifuged at about 1000 *g* for 20 min. and the supernatant fluid used as a standard interferon preparation.

Dosage of heated virus.—In most experiments MEL virus was heated at 56° for 1 hr. in citrate-borate buffer (Methods) and used at a dose of 5 ml. undiluted virus per membrane. However, in experiments to test the effect of virus dosage on yield of interferon it was found that the yield of interferon with 5 ml. of a 1/10 dilution of virus per membrane was almost as good as the yield with undiluted virus. With virus diluted 1/100 there was a sharp decrease in yield. With virus diluted 1/10, the amount of interfering activity produced by the membranes as interferon was about the same as that which the heated virus itself would show in a direct interference test. Fazekas de St. Groth and Edney (1952) noted that the degree of interference produced by heated influenza virus (Lee strain) was maximal when there were roughly 500 agglutinating doses of virus per egg. In the present experiments the maximal yield of interferon occurred when about 800 agglutinating doses of virus per membrane were used.

Conditions of incubation.—When virus-treated membranes were suspended in 50 ml., 100 ml. and 200 ml. of buffer it was found that the yield of interferon in a bottle containing 200 ml. was considerably less than might be expected on the basis of dilution. The large volume increases the violence of agitation but we have not investigated what bearing this factor may have on the yield. However it was found that a bottle containing 50 ml. of buffer kept stationary at 37° gave roughly half the yield of interferon of a similar bottle kept in the shaking machine, so that aeration may play an important part in the yield.

It seemed likely that some metabolic activity was required from the membranes for interferon to be produced. If this were so, one would expect that incubating membranes at 2° instead of 37° would greatly diminish the yield. This was verified in the following experiment. Six chorio-allantoic membranes were incubated with 30 ml. heated MEL for 3 hr. at 37° in the shaking machine. The membranes were then thoroughly washed and incubated without shaking, with

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20 ml. buffer, 3 at 37° and 3 at 2°. After overnight incubation the fluids were harvested and tested for their interferon content. The results are shown in Table I.

TABLE I.—*Titration of Interferon from Membranes Incubated at 2° and 37°*

		Individual haemagglutinin titres of		
Number		Interferon 2°	Interferon 37°	Buffer control
1	.	6	0·5	5·25
2	.	4	<0	6
3	.	6	<0	6·25
4	.	4·5	<0	5
5	.	4·5	<0	5·25
6	.	4·5	<0	5·25
Average per cent of control yield		66	<3	100

Haemagglutinin titres are expressed as $\log_2$ of dilution end-point.

Chorionic and allantoic surfaces.—It is known that the chorionic and allantoic surfaces of the membranes differ in their production of influenza virus and in their behaviour as regards viral interference (Isaacs and Fulton, 1953). It was therefore of interest to see whether application of heated virus to either surface of the membrane would lead to production of interferon.

Groups of 10-day eggs were inoculated with 1 ml. of heated MEL virus per egg (a) by the allantoic route and (b) on the dropped chorionic surface, and in addition a control group of membranes was mixed with the same amount of virus *in vitro* in the standard manner. After 3 hr. incubation the eggs were opened, the membranes removed and washed thoroughly and membranes from all three groups were incubated with buffer overnight at 37°. It was found that interferon of high and comparable activity was produced in all three groups.

Second cycle of interferon production.—It has been previously observed (Isaacs and Lindenmann, 1957) that following incubation of membranes with heated influenza virus the bulk of interferon was liberated between the 3rd and 12th hours. In agreement with this, it was found that whole membranes which had produced interferon in the standard manner when re-suspended in fresh buffer for a second 24 hr. period at 37° did not release more interferon. The possibility was considered that the membranes might have been exhausted of some constituent necessary for the production of interferon. To test this, whole membranes which had liberated a first crop of interferon were mixed with the same dose of heated influenza virus a second time. It was found that these membranes liberated a second crop of interferon which was almost as potent as the first. Such membranes would be expected to show interference on challenge with living virus and it seemed important, therefore, to establish whether production of good yields of interferon was compatible with a highly significant degree of interference. This was tested in the following experiment.

Small pieces of chorio-allantoic membrane were incubated with heated MEL, one piece with 1 ml. of virus per tube. After 3 hr. at 37° the membranes were washed and incubated in 1 ml. amounts of buffer overnight at 37°. Controls received buffer only. After overnight incubation 6 membranes were tested by challenge with MEL virus to see what degree of interference had been established

and it was found that the yield in these membranes was roughly 3 per cent of that of control membranes, *i.e.*, 97 per cent interference was established. At the same time some fluid from these membranes was harvested and it was found that interferon had been liberated. Further groups of these membranes were then incubated a second time with either heated MEL or buffer in the same way, to see whether a second crop of interferon would be produced. The results are shown in Table II.

TABLE II.—*Production of a Second Crop of Interferon*

Group number	1st inoculation	2nd inoculation	HA titre*	Per cent of control yield
1	Heated MEL	Buffer	6·0	71
2	Buffer	Heated MEL	2·0	4·5
3	Heated MEL	Heated MEL	2·9	8·5
4	Buffer	Buffer	6·5	100

* Expressed as mean $\log_2$ of dilution end-point.

The results with group 1 in Table II show that membranes which had produced interferon in the first 24 hr. period did not do so when incubated with buffer in the second 24 hr. period. From group 3 it can be seen that a second inoculation of heated MEL led to just over half the amount of interferon produced in membranes previously treated with buffer (group 2). This yield is out of all proportion to the degree of interference established and it is concluded that production of interferon can occur in membranes in which a high degree of interference with viral synthesis has been produced.

Production of interferon by virus inactivated with U.V. light.—In most experiments we have prepared interferon with virus inactivated by heat, which is a technically simple procedure with large volumes of virus. However, since it is well known that virus inactivated by ultra-violet light is a more efficient interfering agent than virus inactivated by heat (Henle, 1953) it was interesting to see how the two compared as regards interferon production.

In a single experiment in which MEL virus was inactivated by the two methods heated virus at a concentration of 5 ml. per membrane was compared with ultra-violet-inactivated virus at a fifth of this concentration. In spite of the difference in virus dosage the interferon from ultra-violet-inactivated virus was eight times more potent than the heated virus preparation. It was in fact the most potent preparation we had and it is planned to carry out future studies with interferon prepared in this way.

In further experiments the effect of different times of irradiation of the virus was investigated. The membranes used for production of interferon were challenged to establish the degree of interference, and the liberated interferon was titrated in each group. Irradiation for 1, 2 and 4 min. led to the establishment of a high degree of interference in the membranes, with corresponding high yields of interferon. Irradiation for 8 min. gave a significantly lower degree of interference in the membranes while no detectable interferon was produced. However, the test for membrane interference used was more sensitive than the test for the presence of interferon.

The results parallel earlier findings with heated virus (Isaacs and Lindenmann, 1957) ; virus heated at 56° initiated interference and caused production of interferon while virus heated at 60° did neither.

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Action of Interferon

Time of challenge.—In the standard test procedure membranes were kept in contact with interferon for 24 hr. before challenge, a procedure which was based on the fact that interference due to heated influenza virus takes some hours to be established (Fazekas de St. Groth, Isaacs and Edney, 1952). It was found that when the challenge virus was applied along with interferon no interference resulted. In a further experiment in which the interval between introducing interferon and challenge virus was varied, it was found that only slight interference was present with an interval of 6 hr., but there was definite interference at 24 hr. which was not improved by increasing the interval to 48 hr.

Absorption of interferon.—The time interval required for interference to be established might be due to a slow rate of absorption of interferon by the membranes or to the need for some metabolic process lasting a few hours before interference became established. In fact some evidence has been found that both these processes may operate.

In order to measure absorption of interferon a number of tubes were set up each containing 1 ml. of a preparation of interferon and one small piece of chorio-allantoic membrane. These were incubated for 24 hr. at 37° in the roller drum and it was shown that interference was established in the membranes. The fluids were then removed and dialysed against buffer to restore the original salt and glucose concentrations and as a control an aliquot of the original interferon preparation was dialysed at the same time. The interfering activities of these two preparations were then tested ; a comparison between the two indicates the amount of interferon which was absorbed or used up during 24 hr. contact with the membranes. The results are stated in Table III under the heading " first absorption cycle ". It will be seen that the interfering activity was only slightly reduced by 24 hr. contact with the membranes. The experiment was then continued by repeating each experimental step but starting with the first cycle fluid, and in Table III the results are stated under the heading " second absorption cycle ". This shows that a further slight reduction of interfering activity occurred after the second absorption period, but a substantial amount of interfering activity remained unabsorbed. The findings suggest that in this system absorption of interferon by chick chorio-allantoic membranes is not an efficient process.

TABLE III.—*Absorption of Interferon by Living Membranes*

	Buffer control		Interferon after absorption		Interferon controls	
	HA*	Per cent†	HA	Per cent	HA	Per cent
1st absorption cycle 24 hr. . . .	7·3	100	3·3	6	2·8	4
2nd absorption cycle 2 × 24 hr. . .	6·8	100	3·6	11	2·1	4

* mean log$_2$ haemagglutinin titre of 6 tubes.
† per cent control titre.

Effect of temperature on establishment of interference.—The finding that interferon was inefficiently absorbed was surprising since we had earlier found that during a 4 hr. period of contact between interferon and membrane, followed by

20 hr. incubation at 37°, satisfactory interference resulted. However, it was now possible to separate the period of absorption of interferon from its period of action and to test the temperature requirements for the latter.

Four groups of membranes were incubated at 37°, one with buffer and three with interferon. After 3 hr. two of the interferon groups were removed, the membranes washed thoroughly and suspended in buffer. One of these groups was incubated at 37° for 21 hr. and the other group at 2°. The remaining groups were kept for the whole 24 hr. at 37°. At the end of this period, all four groups were challenged with influenza virus in the usual way. The results are shown in Table IV.

TABLE IV.—*The Effect of Temperature on the Establishment of Interference by Interferon*

Group number	Experimental group	HA*	Per cent of control yield
1	Control—buffer	6·9	100
2	Interferon—24 hr. 37°	0·3	1
3	Interferon—3 hr. 37°— Buffer 21 hr. 2°	5·6	41
4	Interferon—3 hr. 37°— Buffer 21 hr. 37°	2·4	4

* Mean $\log_2$ of virus haemagglutinin titre.

The difference between the last groups shown in Table IV indicates that once interferon has been absorbed for a short period of time, further incubation at 37° is still necessary before interference is established.

These experiments on the effect of time of challenge and incubation temperature on the establishment of interference make it clear that interferon acts on the membrane and not on the extracellular virus. Furthermore no effect of interferon on the viral haemagglutinin could be demonstrated.

Dose of challenge virus.—Fazekas de St. Groth and Edney (1952) showed that with heated influenza virus as interfering agent the degree of interference produced was within limits independent of the dose of challenge virus, provided that adequate time was allowed for interference to be established before the challenge virus was introduced. Experiments were carried out with a single dose of interferon given to three groups of membranes and buffer in three control groups. After 24 hr. incubation pairs of interferon and control groups were challenged with a 10^{-3} dilution of MEL virus in the standard way, while other groups received virus diluted 10^{-4} and 10^{-5}. No significant differences were found in the degree of interference in the different groups.

Specificity of interferon.—Attempts were made to prepare interferon from heat-inactivated influenza virus of strains other than MEL, but with virus heated at 56° for 1 hr. the results were very variable. Interferon was therefore prepared from MEL and PR8 strains of influenza virus by ultra-violet irradiation for a standard time and tested for its activity against three different challenge viruses. The results are shown in Table V. The interferon prepared from PR8 was less active than that prepared from MEL when tested against all three strains of challenge virus. Also, Sendai and PR8 viruses were found to be slightly more sensitive to interferon when tested with both preparations. Thus no evidence of specificity of action of interferon was found.

TABLE V.—*Absence of Specificity in Action of Interferon*

Challenge virus	Yield measured as		Interferon from		
			MEL	PR8	*Nil* (control)
MEL .	HA titre*	.	2·4	3·2	6·9
	Per cent of control yield	.	4·5	7·7	100
PR8	HA titre*	.	0·5	2·5	6·8
	Per cent of control yield	.	1·3	5·2	100
Sendai .	HA titre*	.	−1·0	2·2	6·1
	Per cent of control yield	.	<1·5	6·7	100

* Haemagglutinin titre expressed as mean $\log_2$ of a group of 6 tubes.

Effect of interferon in eggs and rabbits.—Interferon is known to interfere with the growth of vaccinia virus in membrane fragments *in vitro* (Isaacs *et al.*, 1957). In order to test whether interferon would act *in ovo*, we prepared a twenty-fold concentrate of interferon by pressure dialysis (see below) and applied 0·1 ml. volumes of this concentrate on dropped chorionic membranes of 11-day eggs. After 24 hr. the eggs were challenged by chorionic inoculation of suitably diluted vaccinia virus and the number of pocks appearing after 48 hr. further incubation was compared with controls. The interference thus established was barely significant. Attempts to produce interference by injecting interferon into the allantoic cavity and challenging with influenza and related viruses likewise failed to show interference. But these experiments will be repeated since, because of the large amounts of interferon needed, we did not try to maintain in the whole egg a relationship between the amount of interferon and the tissue mass comparable to that obtained *in vitro*. When the interferon concentrate was diluted to original volume in normal allantoic fluid instead of buffer and tested by the usual system *in vitro* the expected amount of interference resulted ; thus failure of interferon to act in eggs could not be attributed to its inactivation by some component of normal allantoic fluid.

Heated MEL injected intradermally into rabbits interferes locally with the subsequent development of vaccinial lesions (Depoux and Isaacs, 1954). The same effect could be produced by the intradermal injection of 0·1 ml. of a mixture of ×20 concentrated interferon and a 10^{-5} dilution of vaccinia virus. If the challenge with vaccinia was delayed 24 hr. the effect was less obvious, but this may be due to the difficulty of giving the second injection at exactly the same place as the first. *In vivo* experiments are being continued with more concentrated preparations.

Some Properties of Interferon

Pressure dialysis and freeze-drying.—We had previously found that interferon could be dialysed against buffer without loss of activity (Isaacs *et al.*, 1957). Pressure dialysis proved a convenient way of concentrating interferon up to 10- or 20-fold. When made up with buffer to original volume, such preparations showed no loss of activity. Attempts at a similar concentration by freeze-drying failed. When standard interferon preparations were first dialysed against distilled water, then freeze-dried and made up to volume with buffer, considerable activity could be recovered, but it proved impossible to take up the whole freeze-dried material in small volumes of buffer, since an insoluble precipitate formed which retained substantial amounts of interfering activity.

pH stability.—The pH stability of interferon was tested by dialysing at 2° against large volumes of buffer over the pH range 1–10. After 24 hr. at 2° at the appropriate pH the preparations were dialysed against Earle's solution in order to restore them to their original pH and then tested for their interfering activity. Interferon was stable over the entire pH range.

Treatment with urea.—Attempts were made to dissolve the residue obtained by freeze-drying interferon, previously dialysed against water, in small volumes (1/25th original) of 6M urea. This gave only about a 30 per cent recovery of interferon. It is known that solution of some proteins in 6M urea leads to denaturation sometimes due to molecular dissociation or an increase in molecular asymmetry (Putnam, 1953). However, interferon was stable in 6 M urea and filtration through gradocol membranes showed that the filtration behaviour had not altered (Isaacs *et al.*, 1957). Further, ultra-sonic treatment of interferon (six ½ min. bursts of 350 k.c.) caused no change in filtration behaviour; *i.e.*, complete activity was recovered after filtration through a membrane of average pore diameter 1·8 μ and partial loss of activity occurred with a 1·0 μ membrane.

Miscellaneous.—Interferon was precipitated from solution by saturation with ammonium sulphate and full activity was recovered after addition of the original volume of buffer to the precipitate. Treatment of an interferon preparation (previously dialysed against saline) with sodium periodate ($NaIO_4$) at a final concentration of 0·001, 0·0001 and 0·00001M for 1 hr. at room temperature followed by dialysis against buffer caused no decrease in activity. Shaking with an equal volume of ether at room temperature caused complete loss of activity from ether and aqueous fractions.

Trypsin.—A preparation of interferon was incubated for 2 hr. at 37° with crystalline trypsin at a concentration of 0·01 per cent in Earle's buffered saline. Its interfering activity was greatly reduced by this treatment, and even incubation with 0·001 per cent trypsin considerably reduced its activity, but complete loss of activity was not found.

It seemed possible that the trypsin might have been acting on the membrane rather than the interferon. In order to decide on the site of action of the enzyme, soya bean inhibitor was added (*a*) along with the trypsin (*b*) after incubating interferon with trypsin and (*c*) in a control group without trypsin. The results of an experiment with 0·025 per cent trypsin and 0·025 per cent soya bean inhibitor are shown in Table VI.

TABLE VI.—*Effect of Trypsin on Interferon Action*

Group number	Material tested		$\log_2$ HA titre		Per cent of control yield
1	. Interferon control	.	<0	.	<1·6
2	. Interferon incubated with trypsin	.	3·25	.	15
3	. Interferon incubated with trypsin followed by soya bean inhibitor	.	3·3	.	15
4	. Interferon incubated with soya bean inhibitor mixed with trypsin	.	<0	.	<1·6
5	. Interferon incubated with soya bean inhibitor	.	<0	.	<1·6
6	. Buffer control	.	6·0	.	100

Table VI shows that the trypsin alone partly inactivated interferon, that the effect of the trypsin was completely blocked by mixing it with soya bean inhibitor (group 4) but that the soya bean had no action when it was added to the system after interferon had been incubated with trypsin, but before the chorio-allantoic membranes were added. This shows that the trypsin effect was due to its acting directly on the interferon and not on the membrane.

Other enzymes.—It was difficult to test the effect of various enzymes on interferon unless they could be readily removed from the system or their action inhibited before the membranes were added. The alternative was to incubate interferon for 2 hr. at 37° with low concentrations of enzymes and to use the same concentrations mixed with buffer in the controls. When ribonuclease (0·001 per cent), deoxyribonuclease (0·001 per cent) and the receptor-destroying enzyme of *V. cholerae* (RDE) were used in this way no definite effect on interferon could be demonstrated. In one experiment interferon was incubated with 0·025 per cent trypsin for 2 hr. at 37°, 0·025 per cent soya bean inhibitor was added and it was then incubated with 0·001 per cent ribonuclease for 1 hr. The results are shown in Table VII.

TABLE VII.—*Effect of Trypsin Followed by Ribonuclease on Interferon*

Group number		Material tested		log$_2$ HA titre		Per cent of control yield
1	.	Interferon	.	1·6	.	2·9
2	.	Interferon incubated with trypsin	.	5·3	.	28
3	.	Interferon incubated with trypsin, followed by ribonuclease	.	4·3	.	14
4	.	Buffer control	.	6·7	.	100

It can be seen that trypsin partly inactivated the interferon preparation, and that the ribonuclease did not further inactivate it. The slight depression of virus yield seen in this and in control experiments is probably due to the action of residual ribonuclease on the membranes (LeClerc, 1956).

In one experiment a slight apparent effect of RDE on interferon was observed, but this could not be confirmed in a second experiment. One difficulty is that RDE itself inhibits influenza viral growth in this system and a second difficulty, pointed out by Dr. A. Gottschalk, is that RDE may show variable behaviour from experiment to experiment. In order to overcome these difficulties, interferon was incubated with large doses of live influenza virus (approximately 2500 agglutinating doses of MEL virus/ml. interferon) for 2 hr. at 37° and the virus was effectively removed from the system by addition of viral antiserum and high speed centrifugation followed by further addition of antiserum. Under these conditions no significant action of the viral enzyme on interferon was observed.

DISCUSSION

In its time of appearance following inoculation of virus, and in the presence of an " eclipse phase ", interferon production has shown interesting analogies with virus production (Isaacs and Lindenmann, 1957). However two differences have now emerged. First, interferon was produced in approximately the same

degree whether virus was inoculated on the allantoic or chorionic surfaces of the chorio-allantoic membrane, whereas it is known that the cells on these two surfaces differ considerably in their ability to support multiplication of influenza virus. Secondly, when a large dose of heated virus was incubated with chorio-allantoic membranes interferon was liberated and *interference* was induced, *i.e.*, the membranes showed a greatly reduced ability to support influenza virus multiplication. Nevertheless, these membranes were able to produce a second crop of interferon normally when inoculated with a second dose of heated virus. Both these findings suggest that the whole of the complex machinery required for viral synthesis is not required for production of interferon.

We have already noted that in different systems there was a rough parallel between the degree of interference with viral synthesis induced in membranes and the amount of interferon liberated. In the present study a further correspondence was found, particularly with virus inactivated by ultra-violet light for various periods of time. Interferon is very slowly taken up by chorio-allantoic membranes and incubation of these membranes for some hours at 37° is required before interference is established. This last finding is reminiscent of the fact that interference by heated virus takes some hours to be established even after the virus has been taken up by the cells. The action of interferon also resembles interference by heated virus in two further ways. The degree of interference does not depend on the dose of challenge virus and there is no strain specificity in the action of either agent. Thus although we have no direct evidence that interference is mediated through the action of interferon there is growing circumstantial evidence that this may be the case.

There is little new information on the nature of interferon. Its sensitivity to digestion with trypsin suggests that it may contain protein but there is insufficient evidence to allow any firm conclusions on the presence or absence of nucleic acids, carbohydrate or lipids in combination with the protein. It seems unlikely that we can learn much about its chemical nature until interferon can be prepared in purified form.

SUMMARY

Experimental conditions for preparing interferon in bulk are described. Interferon was produced in similar amounts when heated influenza virus was inoculated on either the allantoic or chorionic surfaces of the chick chorio-allantoic membrane.

Membranes which had produced interferon and which were shown to be unable to support the growth of influenza virus nevertheless produced a second crop of interferon when incubated a second time with heated influenza virus.

Influenza virus inactivated by ultra-violet light gave rise to more potent preparations of interferon than virus inactivated by heat. In these and in other experiments a correspondence was noted between the degree of interference induced and the amount of interferon liberated.

In addition to its action *in vitro*, interferon was found to inhibit the development of pocks by vaccinia virus in the rabbit skin.

Interferon was partly destroyed on incubation with crystalline trypsin.

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