Purification of Interferon from Chick Embryonic Allantoic Fluids and Fibroblast Tissue Infected with Influenza Virus

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SUMMARY
Chick interferon from allantoic fluids of eggs infected with influenza virus and from embryo fibroblast tissue culture was purified to a specific activity of $1.6 \times 10^6$ units per mg. protein; for allantoic fluid interferon this represented nearly 20,000-fold purification. Such material is thought to be still impure. Purified, but not crude, interferon was found to be strictly species specific.

INTRODUCTION
Chick interferon has been purified to varying extents by several groups of investigators. Burke's (1961) starting material was interferon produced in chorioallantoic membranes; this was purified about 20-fold mainly by chromatography on DEAE and SM celluloses. Zemla & Vílcek (1961) precipitated interferon, produced in chick embryo fibroblasts, with acetone or ethanol at $-10^\circ$ to $-15^\circ$ and claimed an 80-fold purification. As their purification factor was however based on total and not protein nitrogen, most of their purification probably consisted in the removal of amino acids and other nitrogenous components of low molecular weight derived from the '199' culture medium. Lampson et al. (1963) purified allantoic fluid interferon about 1800 times (4500 times after electrophoresis) by precipitation of inactive protein with perchloric acid, adsorption of the active material on zinc hydroxide, removal of zinc ions by dialysis in acid solution, chromatography of the active material on CM cellulose and finally electrophoresis on Pevikon. Similar methods were later used by the same authors to purify tissue culture interferon (Lampson et al. 1965). Fantes, O'Neill & Mason (1964) and Fantes (1965), in preliminary communications, described processes that raised the specific activity of chick allantoic-fluid interferon several thousand times. Phillips & Wood (1964) prepared partially purified interferon by fractionation on Sephadex G100. Cantell et al. (1965) and Pokidova et al. (1965) both used methods similar to that of Lampson et al. (1963). Merigan (1964a, b) modified Lampson's method to purify allantoic fluid interferon; he substituted CM Sephadex C25 for CM cellulose and eluted the activity, by employing a pH gradient. An even higher purification (6500-fold) than that achieved by Lampson et al. (1963) was obtained but such material was later shown by electrophoresis (Merigan, Winget & Dixon, 1965) to be still far from homogeneous. This communication describes the isolation of chick interferon of probably even higher specific activity.
Methods

Crude chick interferon

Nine-day-old chick embryos were infected with the B/England/939/59 strain of influenza virus. The allantoic fluids were harvested after 72 hr incubation at 37°. Most of the virus was removed in a Sharples centrifuge at 60,000 g. The supernatant fluid was acidified with HCl to pH 2 to inactivate residual virus; it was neutralized with NaOH after about 16 hr at 4°. Penicillin (100 units/ml.) and streptomycin (100 µg./ml.) were then added, and the fluids were stored at 4° until they were used. They usually contained 1.3 to 2.5 mg./ml. total protein and 40 to 160 interferon units/ml. (defined below).

Interferon was sometimes also prepared from chick embryo monolayer cells, infected with the same strain of influenza virus. Somewhat higher interferon (240 to 320 units/ml.) and lower total protein concentrations (400 to 600 µg./ml.) were obtained.

Adsorbents

Silicates. (1) ‘Doucil-25’ (J. Crosfield and Sons, Warrington, Lancs.) is a synthetic, micronized, amorphous Na-Al-silicate; makers’ specifications: particle size 0 to 25 µ, base exchange capacity 45 ± 2 g. CaO/kg.; SiO₂ 75 %, Al₂O₃ 17 %, Na₂O 8 %. (2) ‘Alusil 165’ (J. Crosfield and Sons); makers’ specifications: ultimate particle size 30 to 50 µ, SiO₂ 78 %, Al₂O₃ 10 %, Na₂O 10 %.

Celluloses. (1) DEAE-cellulose. This was prepared from 25,000 denier rayon, 52 to 100 mesh, at Glaxo Laboratories Ltd., Barnard Castle, Co. Durham (British patent 911,223). It had a capacity of 1.05 m-equiv./mg. (2) CM-cellulose. This was also prepared at Barnard Castle, from the same starting material as the DEAE-cellulose. Its capacity was 0.98 m-equiv./mg.

CM Sephadex. C50, medium grade was used; it was obtained from Pharmacia, Uppsala.

Biological materials

Trypsin. (Grade 1:250; Difco Laboratories, Detroit, Michigan). It was used (0.5 % (w/v) in Earle’s salt solution) in the preparation of chick embryo cell monolayers.

Lactalbumin hydrolysate. (Nutritional Biochemical Corporation, Cleveland, Ohio). It formed (0.25 % (w/v) in Earle’s salt solution) part of the medium in which chick embryo fibroblasts were grown.

Crystalline bovine plasma albumin. (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex). Dilutions (in saline) were used to prepare standard curves for protein assays.

Assay of interferon activity

Preparation of chick embryo cells. Ten-day-old chick embryos (after removal of eyes) were minced by forcing through a perforated stainless steel disc (Porterfield, 1960). The minced tissue was suspended in Earle’s balanced salt solution (approx. 10 ml. per embryo) containing 0.5 % (w/v) trypsin. After stirring for 15 to 20 min. at 37° the suspension was filtered through gauze and then through coarse sintered glass (grade H 1). The filtered cell suspension was diluted with Earle’s solution to a concentration of 3 x 10⁶ cells/ml. To this, calf serum (5 %, v/v) and lactalbumin hydrolysate (0.25 %, w/v) were added.
Purification of chick interferon

Determination of activity. Interferon was serially diluted (usually twofold) in Earle's solution. Duplicate 0.5 ml. portions of cell suspension were mixed in test tubes with 0.5 ml. volumes of the diluted interferon sample. The stoppered tubes were incubated in a sloped position (about 5° off horizontal) for about 16 hr at 37°. Without removing the medium the cell sheets were then challenged with about 100 TCD 50 of Semliki Forest virus in 0.2 ml. of Earle's solution. After about 44 hr at 37° the sheets were examined microscopically for cell damage. The highest (final) dilution of the interferon sample that prevented cytopathic change was taken as the end point; the reciprocal of this dilution will be referred to as the number of interferon units per ml. End points were usually clear-cut and the results were very similar to those obtained in a plaque reduction assay. A standard interferon preparation was always used as an assay control; end points obtained with this material rarely varied by more than half a dilution step. In the early stages, interferon was assayed by a plaque reduction method (also with Semliki Forest virus), but the tube assay was less tedious and capable of dealing with a greater number of samples. Some of the samples were dialysed against Earle's solution before assay to remove cytotoxic substances or to adjust their tonicity.

Protein assay

The method of Lowry et al. (1951) was used. All samples were first precipitated with trichloracetic acid, as interfering substances, especially in the crude materials, tended to give falsely high values. In order to increase sensitivity, cells with a light path greater than 1 cm. were used when necessary. Crystalline bovine plasma albumin was used as the standard.

Screening of potential adsorbents

Slurries of the solid materials in distilled water were sterilized by autoclaving for 15 min. at 10 lb./in.². Measured portions of the suspensions were added to crude interferon to provide final concentrations of the solids of 1 and 10 mg./ml. Samples of the mixtures of either concentration were then adjusted to pH values of 3, 6 and 9 and shaken mechanically for 1 hr at room temperature. The solids were removed on a centrifuge and the decanted neutralized supernatant fluids were assayed for interferon and total protein content. Whenever appreciable adsorption of interferon had occurred, the sedimented solids were suspended in 0.7 M-phosphate buffer pH 7.5, with (in initial experiments) a volume equal to that of the original interferon solution. These suspensions were again shaken and centrifuged and their supernatant fluids dialysed and assayed for interferon content.

Precipitations with methanol

At pH 7.5. Methanol (5 vol.:vol.) was added slowly at 4° or at room temperature. The precipitated protein was collected by centrifugation (room temperature, 1000 to 1500 g, 10 min.) after 16 hr storage at 4°.

At pH 2. The aqueous solution was adjusted to pH 2 with HCl; methanol (5 vol.:vol.) was added as described above, and the inert protein precipitate was discarded after 16 hr standing at 4°. The pH of the supernatant aqueous methanol was adjusted to an apparent value of 7.5 as measured on a glass electrode; the precipitate containing interferon was collected after 16 hr at 4° and was then extracted with 0.01 M-phosphate
buffer pH 7.5. The solutions could then be chromatographed directly (without dialysis) on DEAE-cellulose columns.

**DEAE-cellulose chromatography**

Columns were prepared in the normal way. The ion exchanger was treated with 0.5 N-NaOH, water, 0.5 M-phosphate buffer pH 7.5 and then water again. It was finally equilibrated with 0.01 M-phosphate buffer pH 7.5. The natural flow rate was fairly fast, but was usually restricted to about 4 to 6 ml./hr for every g. of exchanger.

**CM Sephadex chromatography**

Columns prepared from this material were treated with 0.5 N-NaOH, water, 0.5 M-HCl and water until neutral; they were finally equilibrated with 0.1 M-phosphate buffer pH 5.8. A flow rate of about 30 to 60 ml./hr for every 500 mg. of exchanger was employed.

Activity was adsorbed from 0.1 M-phosphate solution pH 5.8. Columns were then washed with 0.1 M-phosphate buffer pH 6.0 until the protein concentration of the effluent was only 1 to 2 µg./ml. Elution of interferon was done in the first place by a pH gradient (Merigan, 1964b). This was usually formed by passing 0.1 M-phosphate buffer pH 8.3 into a constant volume mixing vessel containing 0.1 M-phosphate buffer pH 6.0. When steeper gradients were required pH 9.5 or even pH 12.3 buffers were used. The dimensions were generally chosen to produce fairly shallow gradients. The most highly purified interferon fractions could be further concentrated by readsoption on another CM Sephadex column and direct elution with 0.1 M-phosphate buffer at pH 6.4 to 6.6. Pervaporation at 4 ° could be used as an alternative means of concentration. The method was similar to that of Polson & Hampton (1957).

**RESULTS**

**Screening of potential adsorbents**

The first object was to reduce the volume of crude fluid and thus make subsequent purification steps more manageable. Simultaneous purification was another desirable objective. Ammonium sulphate precipitation, as used by, for example, Burke (1961), was rather messy and also unselective; moreover it was frequently found that some of the active precipitated protein floated in the high-density salt solution and would not settle, even on centrifugation.

Many materials were tested. Some were unsuitable because they adsorbed neither protein nor interferon to any great extent; others adsorbed interferon irreversibly and a third group was entirely nonselective. The following substances were unsuitable for one or other of these reasons: AlPO₄, CaHPO₄, Ca₃(PO₄)₂, Al(OH)₃, Zn(OH)₂, Pb(OH)₂, Cu(OH)₂, Ba(OH)₂, Fe(OH)₂, Fe(OH)₃, Cd(OH)₂, Co(OH)₂, Ni(OH), chalk, BaSO₄, Al₂O₃, SiO₂, Mg-trisilicate, Al-F-silicate, talc, pumice, kieselguhr, ultramarine, flowers of sulphur, vermiculite, glass powder, fullers earth, kaolin, bentonite, various charcoals, Decolorite (Permutit Co., London, W.4), nylon powder, Ca-silicate and various micronized silicas.

Low concentrations (0.1 to 0.2 mg./ml.) of freshly prepared Al(OH)₃, sometimes removed appreciable amounts of inert protein, leaving partly purified interferon in the
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supernatant fluid. However, this was not reproducible and interferon was occasionally carried down by the adsorbent.

Bentonite adsorbed interferon readily but elution was only partial (Wagner, 1960)
The Na-Al-silicates ‘Doucil’ and ‘Alusil’, at a final concentration of 4 to 5 mg./ml. (3 to 3.5 mg./ml. for tissue culture interferon), adsorbed at pH 5 all interferon and about half the total protein. Treatment of the adsorbates with 0.7 M-phosphate buffer pH 7.5 (0.1 to 0.2 vol. of the original interferon solutions) eluted most or all interferon and some 20% of the total protein. A five- to tenfold concentration by volume with a three- to fivefold increase in specific activity was thus achieved. Doucil was used in most of the experiments.

Elution of interferon from Doucil with solutions other than phosphate

The high phosphate content of the eluates was rather inconvenient when further purification steps were attempted. Adsorption on ion exchangers had to be preceded by dialysis, and precipitation of interferon with methanol, ethanol or acetone was accompanied by precipitation of the buffer salt. In order to overcome this disadvantage, aqueous solutions of Na-acetate or pyridine or mixtures of the two were tried, but the elution efficiency was found to be low. Interferon was eluted rather more efficiently at pH 8 by solutions of ammonium formate or bicarbonate, and interferon almost salt free could be recovered from such solutions by freeze-drying.

Use of iodide or thiocyanate

Aoki & Hori (1962) discovered that albumin was precipitated when solutions were acidified in the presence of iodide or thiocyanate. We found that the addition to crude interferon of KSCN to give final concentrations of 0.4 to 1.0 M and then acidification with HCl to give pH values of 3 to 3.5 precipitated 65% to 85% of the inert protein. More inert protein was precipitated from solutions of higher salt concentrations and lower pH values, but at 1 M-KSCN and pH 3 some interferon was carried down with the protein precipitate. It was better to perform the precipitation in two stages, acidifying to pH 4 first, removing precipitated protein and then acidifying further to pH 3. KI behaved similarly, though solutions of slightly higher molarity were needed to achieve the same degree of purification. When interferon was precipitated by methanol (5 vol. at pH 7.5) KSCN, unlike phosphate, stayed in solution.

Interferon adsorbed to Doucil could be eluted with 0.5 M-KSCN at pH 7.5 instead of with phosphate buffer. Such eluates (1/5 to 1/10 the starting volume) were then acidified with HCl to pH 3.5 (or to pH 4 and then to pH 3); the supernatant fluids, after removal of precipitated inert protein by centrifugation (1000 to 1500 g, 10 min.) contained 50% to 90% of the original interferon, concentrated 5 to 10 times by volume with specific activity increased ten- to 40-fold (Table 1).

Precipitation of interferon with organic solvents

The addition of 2 to 3 vol. of acetone or 5 vol. methanol to crude, neutral interferon solutions precipitated all activity and most or all of the inert proteins. The precipitates could be redissolved in smaller volumes of Earle’s solution or phosphate buffer. This concentrated interferon, but did not purify it to any extent. However, when methanol or acetone was added to the neutralized KSCN eluates from Doucil, some of the inert precipitated proteins were denatured and would not redissolve; additional purifica-
tion was thus achieved and not infrequently interferon with a specific activity more
than 100-fold that of the crude material was obtained. By redissolving the interferon
from such precipitates in small volumes of buffer, it was possible to achieve up to
500-fold concentrations by vol. The overall recovery of interferon up to this stage
varied between 30% and 80% and that of total protein between 0·2% and 1·5%.

Table 1. Initial purification steps

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume (ml.)</th>
<th>Interferon (units/ml.)</th>
<th>Protein (μg./ml.)</th>
<th>Interferon (units/mg. protein)</th>
<th>Purification factor</th>
<th>Recovery of Interferon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude interferon (allantoic fluid)</td>
<td>200</td>
<td>60</td>
<td>1310</td>
<td>46</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Doucil eluate (0·5 M-KSCN, pH 7·5)</td>
<td>40</td>
<td>200</td>
<td>1530</td>
<td>130</td>
<td>2·8</td>
<td>67</td>
</tr>
<tr>
<td>Doucil eluate (pH 3·5 supernatant fluid)</td>
<td>40</td>
<td>200</td>
<td>225</td>
<td>890</td>
<td>19</td>
<td>67</td>
</tr>
</tbody>
</table>

The solubility of interferon in acidified aqueous solvents

Interferon and most extraneous protein were precipitated by trichloracetic acid (2·5% to 5%, w/v); some inert protein but little or no interferon was precipitated with 1% of this reagent. Trichloracetic acid thus resembles perchloric acid in its effect on interferon solutions (Lampson et al. 1963). On redissolving the precipitates resulting from the higher concentrations of trichloracetic acid, most of the interferon was recovered, but little purification occurred in this way. When attempts were made to remove trichloracetic acid from precipitates by washing with aqueous lower alcohols or acetone, it was found that most of the interferon activity disappeared from the precipitates, but it could usually be shown to be present in the dialysed washing fluids. Interferon behaved like albumin in this respect (Michael, 1962). When a mixture of 10 vol. of methanol and 0·02 vol. of concentrated HCl was added to 1 vol. of crude chick interferon, a precipitate of inert protein resulted. On neutralizing the aqueous methanolic supernatant solution with NaOH, interferon, purified about two- or threefold, was precipitated. In another experiment it was shown that the addition of 3 to 9 vol. of methanol, or 2 to 3 vol. of acetone, to crude interferon previously adjusted to pH 2 with HCl only precipitated inert protein. The interferon was found in the acidic aqueous organic solvent phase, where it was assayed after removal of the solvent by dialysis against Earle's solution. The addition of these solvents at pH values 3 to 9, however, resulted in the partial or total precipitation of interferon. By making use of the solubility of interferon in acidic aqueous methanol, the purification steps described under the preceding heading were modified (Fantes, 1965). Instead of neutralizing the acidified 0·5 M-KSCN eluate before the addition of methanol, the pH was further reduced to 2, and the precipitate was discarded. Methanol (5 vol.) was then added, another inert precipitate was removed, the pH of the supernatant fluid was adjusted with NaOH to an apparent value of 7·5, and the resulting interferon-containing precipitate was extracted with a small volume of 0·01 m-phosphate buffer pH 7·5.
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Chromatography of partly purified interferon on DEAE-cellulose

The 0.01 M-phosphate buffer pH 7.5 solution was passed through a DEAE-cellulose column of the same pH and molarity. When the ion exchanger/interferon ratio was chosen correctly, most or all of the activity could be eluted with the same buffer; 10 to 40 interferon units per mg. DEAE-cellulose proved to be the optimal loading. At this (pre-DEAE) stage such an amount of interferon was usually associated with 2 to 10 \( \mu \)g. of protein. When the number of units per mg. of ion exchanger was much less than 10, the recovery of interferon became progressively poorer. If the column was overloaded, less purification was achieved. With correct loading most of the eluate fractions contained over 30,000 interferon units per mg. protein, the best fractions about 300,000.

Concentration and chromatography of dilute, partly purified interferon on CM Sephadex

Chromatography on DEAE-cellulose caused considerable dilution of the concentrated interferon; attempts to concentrate it with zinc acetate or CM cellulose (Lampson et al. 1963) were not very successful, but CM Sephadex (Merigan, 1964a, b) proved more suitable for this purpose. To the dilute solutions strong phosphate buffer pH 5.8 was added to a final concentration of 0.1 M. The solutions were then passed slowly through a small CM Sephadex column of the same pH and molarity, with 1 mg. of the ion exchanger for every 100 to 2000 interferon units. Under these conditions the interferon was retained on the column, which was then washed with 0.1 M-phosphate buffer pH 6.0 until the protein concentration of the effluent fell to 1 to

Table 2. Influence of steepness of pH gradient and of relative weights of CM Sephadex on the pH value at which peak activity is eluted

<table>
<thead>
<tr>
<th>Example</th>
<th>Weight of CM Sephadex (mg.)</th>
<th>Capacity of mixing vessel (ml.)</th>
<th>Buffers used in gradient (pH/pH)</th>
<th>Specific activity of sample ( \times 10^6 ) (units/mg. protein)</th>
<th>Loading of CM Sephadex (units/mg.)</th>
<th>pH of elution peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>530</td>
<td>6/8.3</td>
<td>20</td>
<td>600</td>
<td>6.25</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>280</td>
<td>6/12.3</td>
<td>103</td>
<td>1800</td>
<td>7.0</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>280</td>
<td>6/9.5</td>
<td>25</td>
<td>700</td>
<td>6.35</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>280</td>
<td>6/9.5</td>
<td>62</td>
<td>90</td>
<td>6.75</td>
</tr>
</tbody>
</table>

See text for formation of gradients. Eluates were collected in 5 ml. portions and the pH and interferon content of each was determined.

2 \( \mu \)g./ml.; traces of activity were sometimes also found in these washings. The interferon was eluted by means of a pH gradient. Activity started to emerge from the columns at pH 6.1 to 6.4; at about pH 6.9 most interferon was usually eluted, but occasionally appreciable activity was still observed at much higher pH values. The pH at which, in various experiments, peak activity emerged (pH 6.25 up to about pH 7.2) was greatly influenced by two factors. The steeper the pH gradient for similar weights of CM Sephadex (examples 1 and 2, Table 2) and the larger the weight of CM Sephadex for a given gradient (examples 3 and 4, Table 2), the higher was the pH at which peak activity was found.

These observations suggest that ionic binding is not the only force that governs the
behaviour of chick interferon on CM Sephadex C-50, but that retardation due to lattice penetration also plays an important part. One might expect chromatography on the smaller pore C-25 grade to be influenced to a lesser extent by molecular sieving. For isoelectric point determinations (Lampson et al. 1965; Merigan et al. 1965) both grades should be suitable but only if the buffer volumes used for the individual elution steps are sufficiently large for equilibrium conditions to be reached.

Although the figures in the table suggested a possible correlation between the pH of the peak activity and the purity of the sample, other results did not support this. We have some preliminary evidence that the active material may not be entirely homogeneous; this was, however, not the only reason for the diverse elution behaviour, since in another experiment portions from the same sample, adsorbed on identical columns but eluted with two different pH gradients, gave peak activities at different pH values. By choosing shallow gradients and collecting the eluates in small volumes (usually 5 ml.), fractions of very high specific activities could be obtained.

The most highly purified fractions (often > 10^6 units/mg. protein) were sometimes further concentrated by pervaporation at 4°, or by readsorption on a CM Sephadex column (0.1 M, pH 5.9) and direct elution with 0.1 M-phosphate buffer pH 6.4 to 6.6, the latter process sometimes affording further slight purification but usually also causing some loss of activity. The most highly purified samples so far obtained from either source had a specific activity of 1·6×10^6 units/mg. protein, representing a nearly 20,000-fold purification of allantoic fluid interferon (specific activity 85 units/mg. protein). Material that had not been previously passed through DEAE-cellulose contained some inert protein with an elution peak at a pH value only just higher than that of interferon. It was therefore possible to obtain only little, if any, of the interferon free from this contaminating protein. The purification of crude tissue culture interferon by the process finally adopted is shown in Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume (ml.)</th>
<th>Total units (× 10^6)</th>
<th>Specific activity*</th>
<th>Purification factor†</th>
<th>Overall recovery of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude culture fluid</td>
<td>22,500</td>
<td>5·40</td>
<td>470</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>pH 7·5, 0·5 M-KSCN</td>
<td>3,000</td>
<td>4·80</td>
<td>1,980</td>
<td>4·2</td>
<td>89</td>
</tr>
<tr>
<td>Doucil eluate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 3·5 supernatant fluid</td>
<td>3,000</td>
<td>3·60</td>
<td>5,180</td>
<td>11</td>
<td>67</td>
</tr>
<tr>
<td>pH 2·0 supernatant fluid</td>
<td>3,000</td>
<td>2·37</td>
<td>15,500</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>Redissolved methanol (pH 7·5)</td>
<td>225</td>
<td>1·80</td>
<td>19,300</td>
<td>41</td>
<td>33</td>
</tr>
<tr>
<td>precipitate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined DEAE-cellulose eluate</td>
<td>960</td>
<td>1·14</td>
<td>132,000</td>
<td>281</td>
<td>21</td>
</tr>
<tr>
<td>fractions (combined peak fractions)</td>
<td>25</td>
<td>0·40</td>
<td>1,600,000</td>
<td>3,400</td>
<td>7</td>
</tr>
</tbody>
</table>

*Number of interferon units per mg. protein.
†Specific activity of sample / Specific activity of starting fluid

Stability of highly purified interferon on storage

Unlike Merigan (1964b) and Lampson et al (1963) we found that some of the activity of highly purified interferon was always lost on storage, whether it was kept frozen or at 4°, even in polypropylene containers.
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Homogeneity of purified interferon

Earlier results obtained on electrophoresis of highly purified chick interferon in polyacrylamide gel (Williams: see Fantes, 1965; Fantes, 1966a) or in a sucrose gradient (Polson: see Fantes 1966b) suggested that the samples were substantially homogeneous. More recent work, however, has shown that even the most highly purified material so far obtained is probably still impure. It is hoped to publish these findings in more detail later.

Isoelectric point

A sample of partly purified interferon was subjected to zone electrophoresis in a sucrose gradient by Dr A. Polson (1965, personal communication), at the University of Cape Town. Its mobility suggested an isoelectric point of about pH 6.8, a value in broad agreement with that accepted by several other workers (Bodo & Jungwirth 1964; Lampson et al. 1965; Merigan et al. 1965).

Species specificity of purified interferon

We have already reported that purified and concentrated chick interferon was species specific (Fantes et al. 1964). Since then Baron, Barban & Buckler (1964) and Merigan (1964a) have also shown that purified chick interferon of high potency in chick cells did not protect heterologous cells against viral attack. Our own partially purified interferon (pre-DEAE material), containing 2500 chick units per ml, was found to be inactive when tested in vervet monkey kidney cells against influenza virus (KUNZ strain), Sabin’s type 2 polio virus, vaccinia virus or the bovine M6 virus. A comparison of crude allantoic fluid interferon (chick titre 1/120) with the partly purified and concentrated material (chick titre, 1/2500) showed that the former had some activity against the rhinovirus HGP in human embryonic lung cells (R. D. Andrews, 1964, personal communication), against vaccinia virus in the rabbit skin (R. D. Andrews, 1964, personal communication) and against parainfluenza virus type 1 (Sendastrain) in L cells (N. B. Finter, 1964, personal communication), whereas the partly purified material had no activity in these systems. Since the purified interferon was about 20 times more potent in chick cells than the crude interferon, it is clear that the heterologous activity was not due to chick interferon, but to something else.

Following the claim of the Olin Mathieson Chemical Corporation (1962; U.S.P. 195, 428) we tried to prepare by a similar method “non-specific” interferon from two samples of partly purified chick interferon (specific activities 1600 and 12,000 units per mg. protein). Incubation of the interferon at pH 7.5 for 3 hr at 37° with 20 and 200 µg/ml. carboxypeptidase A (1000 units/mg., Koch-Light Laboratories, Colnbrook, Bucks., England) had no effect on potency in chick cells, but the treatment failed to produce any measurable (< 1/2) activity in monkey kidney cells (against Sabin type 2 polio virus) or in rabbit kidney cells (against the bovine M6 virus).

Some other biological and physico-chemical properties of chick interferon, purified by methods described in this paper, have already been reported (Fantes & O’Neill, 1964; Fantes & Furminger, 1965).
DISCUSSION

Methods previously used by us to purify crude chick interferon from influenza virus-infected allantoic fluid (Fantes et al. 1964; Fantes 1965) could also be used for purifying tissue culture interferon. The most highly purified material so far obtained by us from both sources contained $1.6 \times 10^6$ units per mg. protein. As this specific activity was higher than any yet reported by other workers, we wished to verify that the difference was not due to different sensitivities of the assay methods. Therefore, identical freeze-dried samples (kindly supplied by Dr T. C. Merigan) were assayed concurrently in Dr Merigan’s and in our own laboratories; the results obtained by the two methods were very similar.

Although the most highly purified interferon so far obtained is probably not pure, the high level of biological activity of even the present material (viral multiplication is prevented by less than $10^{-6}$ mg. protein/ml.) is exceeded only by a few vitamins and antibiotics. Other manifestations of the very small amount of protein associated with considerable biological activity were perhaps the difficulties encountered in preparing an antiserum to interferon (Paucker & Cantell, 1962) and the more recent failure to find an interferon messenger RNA (Burke & Walters, 1966).

Little is known about the mechanism by which interferon renders cells resistant to viral attack. Several misleading results have been obtained in attempts to unravel, using crude interferon, this complex chain of events and it is probable that only highly purified interferon will lead to further progress in this field (Sonnabend & Friedman, 1966).

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REFERENCES


Purification of chick interferon


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