Purification of Human Fibroblast Interferon by Zinc Chelate Chromatography

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SUMMARY

Human interferon was prepared by superinduction of cultures of either diploid embryonic skin and muscle cells or of the osteosarcoma cell line MG-63. The interferon so obtained was concentrated and partially purified by adsorption to controlled pore glass (CPG) beads at neutral pH and desorption by glycine-HCl buffer at pH 2. After neutralization, this interferon was applied to a column of zinc chelate which was eluted with buffers of decreasing pH. Most of the proteins eluted ahead of the interferon activity, which itself eluted in two distinct peaks. The first peak occurred in the effluent fractions around pH 5.9, and the second one in fractions around pH 5.2. The interferon found in fractions of pH 5.9 contained 5% of the original contaminating proteins. In contrast, the amount of total protein in the pH 5.2 peak was so small that it could not accurately be assayed by the fluorescamine method. Consequently, the interferon in the peak fraction was estimated to have a specific activity of about $2 \times 10^9$ units/mg. This material was radiolabelled and analysed by electrophoresis. A major peak of about 22000 mol. wt. with only minor contaminating proteins appeared on the autoradiographs. The total recovery of the zinc chelate chromatographical procedure was nearly 100%, and the interferon recovered from each peak behaved consistently on rechromatography.

Fibroblast interferon produced by most diploid cells contained less than 10% of the variant eluting at pH 5.9. MG-63 cells and high-passage cultures of some diploid cell strains produced up to 50% of this variant.

INTRODUCTION

In the human species, three distinct types of interferons have been described. A recently proposed nomenclature (International Committee, 1980) for these molecular types is HuIFN-α (formerly human leukocyte-type interferon), HuIFN-β (formerly human fibroblast-type interferon) and HuIFN-γ (formerly human immune interferon). HuIFN-β is usually prepared by treating cultured human diploid fibroblasts with double-stranded polynucleotides, polyriboinosinic-polyriboctydyllic acid [poly(rI).poly(rC)] in conjunction with the metabolic inhibitors cycloheximide and actinomycin D (Havell & Vilcek, 1972; Billiau et al., 1973). The preparation so obtained is called fibroblast interferon; the interferon molecules present are exclusively of the HuIFN-β type.

Various techniques, as reviewed by Knight (1978), have been described to achieve partial purification of fibroblast interferon. Knight (1976) was the first to report apparently complete purification using a sequence of various procedures, each accompanied by a certain loss in biological activity. The overall recovery was about 10% and the specific activity of the
end-product was estimated at $2 \times 10^8$ units/mg protein. Similar multi-step procedures for the purification of fibroblast interferon were used by other authors (Berthold et al., 1978). In subsequent studies Knight et al. (1980) used adsorption to Blue Sepharose and desorption with ethylene glycol, and achieved purification to a specific activity of $5 \times 10^7$ units/mg protein in only two stages with a recovery of 50% of the initial biological activity. Subsequent electrophoresis in gels containing sodium dodecyl sulphate (SDS) allowed purification to homogeneity (sp. act. of $2 \times 10^8$ to $8 \times 10^8$ units/mg protein) with a recovery of 5 to 20%. This material was used for the determination of amino acid composition and of amino-terminal amino acid sequence (Knight et al., 1980). The results of this study were subsequently found to correspond to the nucleotide sequence of cloned genes of HuIFN-$\beta$ (Derynck et al., 1980; Taniguchi et al., 1980).

From the characterization studies there is no evidence for polymorphism in the primary structure of HuIFN-$\beta$. However, analysis by isoelectric focusing has suggested the existence of distinct molecular populations of HuIFN-$\beta$ with different charge density, presumably due to quantitative or qualitative differences in glycosylation (Morser et al., 1978).

In the present paper we describe a method for purification of human fibroblast interferon to a specific activity of about $2 \times 10^8$ units/mg protein, in two technically simple steps, with a total recovery of about 50%. In addition, this method allowed the separation of certain preparations of fibroblast interferon into two apparent subpopulations.

**METHODS**

**Cell cultures.** The origin of the human diploid embryonic skin muscle fibroblast strains, E$_{SM}$ and E$_{SM}$ and the osteosarcoma cell line MG-63 were described earlier (Billiau et al., 1977, 1979). HEp-2 cells were obtained from the American Type Culture Collection (strain CCL23).

**Production of fibroblast interferons.** Interferon was produced by applying the priming and superinduction schedule (Havell & Vilcek, 1972; Billiau et al., 1973) to E$_{SM}$, E$_{SM}$ or MG-63 cells, cultivated in roller bottles. The technology for large scale production has been described in detail in previous papers (Billiau et al., 1979). The yield with these techniques was about 30 units per 1000 cells in the case of E$_{SM}$ or E$_{SM}$ cells and about 120 units per 1000 cells for MG-63 cells. The crude material had a titre of about $10^5$ units/ml with a specific activity of $10^4$ to $10^5$ units/mg protein, the main contaminant being the proteins present in the human plasma protein that was used as a protein replacement in the culture medium during the production phase.

**Titration of interferon.** Interferon titrations were done with a cytopathic effect-inhibition microtitre assay on HEp-2 cells using vesicular stomatitis virus as a challenge and crystal violet to stain the cells (Volekaert-Vervliet & Billiau, 1977). All values were corrected against the international standard of fibroblast interferon (G-023-902 527), said to contain 10000 international units/ampoule. When reconstituted in 1 ml of fluid this standard preparation had an endpoint, in our method, at dilution $10^{-3.0}$ to $10^{-3.3}$.

**Purification methodology.** All interferon used in the present study first underwent partial purification by CPG adsorption. The procedure is described in detail in previous papers (Edy et al., 1976; Billiau et al., 1979); it is summarized in the protocol outline shown in Fig. 1. The interferon was subsequently chromatographed on zinc chelate columns, using a method modified from Edy et al. (1977). The column beads were prepared by chelating zinc to iminodiacetate (Aldrich Europe, Beerse, Belgium) immobilized on epoxy-activated Sepharose 6B (Pharmacia) as described by Porath et al. (1975). After use, the columns were regenerated. First they were stripped by washing with 5 bed vol. 0.05 M-EDTA in NaCl-phosphate pH 7.4 (1 M-NaCl, 0.02 M-phosphate pH 7.4). The EDTA was removed by washing with an equal volume of the same buffer. The columns were then equilibrated with NaCl-acetate pH 4.
Purification of human fibroblast interferon

I. Controlled pore glass (CPG) purification

Mix 1 vol. CPG beads with 30 vol. crude human fibroblast interferon and stir for 2 h.

*Wash* 2×

1 vol. CPG with 10 vol. PBS

*Wash* 1×

1 vol. CPG with 10 vol. 0.01 M glycine–HCl buffer pH 3.5

*Elute* 2×

Stir 1 vol. CPG with 1 vol. of above buffer for 5 min

*Elute* 2×

Stir 1 vol. CPG with 1 vol. of above buffer for 30 min

*Elute 2×*

Stir 1 vol. CPG with 1 vol. of above buffer for 30 min

*Dialyse*

Pool eluates and dialyse against a sufficient vol. of NaCl–phosphate pH 7.4 to reduce the glycine concentration from 0.3 M to 0.1 μM

II. Zinc chelate chromatography

Apply sample at 15 to 20 ml/h

Sample: 5 to 50 ml

100 to 400 ml

Column: 0.9 × 8 cm (K 9/15; Pharmacia)

1.5 × 16 cm (K 15/30; Pharmacia)

*Wash* with 1.5 bed vol. NaCl–phosphate pH 7.4

*Wash* with 5 bed vol. NaCl–phosphate pH 5.9

*Elute*

Initially with 1 bed vol. NaCl–acetate pH 4.2, followed by 2 bed vol. of same buffer pH 4

Collect 1 ml fractions and determine pH

Pool pH range 5.6 to 4.2 which contains 98% of purified human fibroblast interferon

Fig. 1. Purification procedure for human fibroblast interferon. All steps were performed in the cold (4 °C).
polyacrylamide gel cross-linked with N,N'-diallyl-tartardiamide (Anker, 1970), and a 3% stacking gel. The gels were prepared for fluorography and exposed to X-ray film (Curix RP₂, Agfa Gevaert, Mortsel, Belgium) at -70 °C (De Ley et al., 1979).

**Antiserum.** An antiserum against interferon was prepared by injecting a goat with six consecutive weekly intramuscular injections of 10 × 10⁶ units fibroblast interferon purified by CPG adsorption and zinc chelate chromatography (fraction eluting at pH 5-2). To avoid as much as possible the formation of antibody to foreign proteins, the material was stabilized by addition of the goat's own pre-immunization serum. For the first five injections incomplete Freund's adjuvant was used. The final injection was with complete adjuvant. The goat was bled 11 days later. The serum partially neutralized 10 units/ml interferon at a dilution of 1/200,000 to 1/400,000.

**Human plasma protein fraction (HPPF).** HPPF, Cohn fraction V, was obtained as a sterile, pyrogen-free solution containing 45 mg protein/ml (Belgian Red Cross National Blood Transfusion Service).

**Determination of protein concentrations.** Protein concentrations in column fractions were determined using the fluorimetric method described by Böhlen et al. (1973). Briefly, 100 µl of sample was diluted in 2 ml 0.1 M-sodium phosphate buffer pH 7.4 to which 700 µl fluorescamine (Flurame; Serva, Heidelberg, F.R.G.) stock solution (6 mg in 20 ml 1,4-dioxane) were added. After 10 min incubation at 20 °C, fluorescence was measured in a fluorescence spectrophotometer (Perkin Elmer, model 1000, Ueberlingen, F.R.G.) at 480 nm wavelength. Serial dilutions of a standard solution of crystalline bovine serum albumin were run in parallel. In control experiments, it was found that this procedure allowed us to accurately determine the protein concentration in samples containing >5 µg/ml. Twofold differences in readings on samples containing <2.5 µg/ml were probably not meaningful.

**RESULTS**

**Zinc chelate chromatography of human fibroblast interferon from diploid cells**

Human fibroblast interferon from diploid E₁SM cells (prepurified by adsorption to CPG) was chromatographed on a zinc chelate column. An example of this type of experiment is shown in Fig. 2. A sample of 5 ml interferon, containing 5 × 10⁴ units, was dialysed against NaCl–phosphate pH 7.4 and applied to the column at 4 °C. The column was washed first with NaCl–phosphate pH 7.4 and second with NaCl–acetate pH 5.9. It was then developed by a pH 6 to 4 gradient in NaCl–acetate. Under these conditions, proteins, as measured by fluorimetry, eluted in two peaks: a major peak in fractions of pH 7 (first column wash) and a minor peak in fractions between pH 7 and 5.9 (second column wash). No interferon was found in the major protein peak, a small amount was recovered in the minor protein peak and even less in subsequent fractions preceding the gradient. The bulk of interferon (>90% of the total amount eluted) was recovered in fractions corresponding to the pH gradient between pH 5.2 and 4.2. Since no protein was detectable under this interferon peak, it was decided to scale up the method in order to be able to determine the specific activity and homogeneity of this material. Also, the possibility was considered that the combination of CPG adsorption and zinc chelate chromatography would constitute a simple method for the complete purification of fibroblast interferon.

**Two-step purification of diploid fibroblast interferon to specific activity of >2 × 10⁹ units/mg protein**

For large scale purposes the zinc chelate chromatography described above was slightly modified in that the final development of the column was performed with NaCl–acetate pH 4.2 and pH 4 buffers instead of a pH 6 to 4 gradient. A complete protocol outline starting
Purification of human fibroblast interferon

Fig. 2. Chromatography of human fibroblast interferon on zinc chelate agarose. Five ml CPG-purified human fibroblast interferon with a titre of $10^5$ units/ml were dialysed against NaCl-phosphate pH 7-4, and applied to a 0.9 x 8 cm column (K 9/15, Pharmacia) at 4 °C (15 to 20 ml/h). The column was washed with 25 ml NaCl-acetate pH 6, followed by the pH gradient. The gradient was developed by mixing 20 ml NaCl-acetate buffer at pH 6 and pH 4. Arrows indicate time of addition of buffer. ●, Protein concentration; ▼, interferon concentration; O, pH.

Table 1. Large scale purification of human fibroblast interferon in two steps: by CPG adsorption and zinc chelate chromatography

<table>
<thead>
<tr>
<th>Designation of fractions</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (units x $10^6$)</th>
<th>Specific activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPG purification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>3200</td>
<td>1075.2</td>
<td>64</td>
<td>$5.9 \times 10^4$</td>
</tr>
<tr>
<td>Unadsorbed</td>
<td>3200</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wash</td>
<td>1500</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Eluate I</td>
<td>140</td>
<td>124.6</td>
<td>36.8</td>
<td>$2.96 \times 10^5$</td>
</tr>
<tr>
<td>Eluate II</td>
<td>140</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
<td></td>
<td>57.5%</td>
</tr>
<tr>
<td>Zinc chelate chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysed fraction of eluate pool</td>
<td>100</td>
<td>44.46</td>
<td>13.2</td>
<td>$2.96 \times 10^5$</td>
</tr>
<tr>
<td>Void + pH 7.4 wash</td>
<td>130</td>
<td>42.51</td>
<td>&lt;0.0002</td>
<td>—</td>
</tr>
<tr>
<td>pH 5.9 wash</td>
<td>150</td>
<td>1.60</td>
<td>0.76</td>
<td>$0.47 \times 10^6$</td>
</tr>
<tr>
<td>pH 5.2 eluate*</td>
<td>6</td>
<td>~0.007</td>
<td>12.02</td>
<td>~$1.7 \times 10^9$</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
<td></td>
<td>91.4%</td>
</tr>
<tr>
<td>Overall recovery</td>
<td></td>
<td></td>
<td></td>
<td>52.6%</td>
</tr>
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</table>

* Pool of fractions eluting between pH 5.6 and 4.2.

from crude interferon is presented in Fig. 1, and Table 1 shows the results obtained in a representative experiment. The overall recovery was 52.6% of the initial biological activity. Over 18 runs, the recovery of the zinc chelate column only, averaged 69.5% (s.e. 6.62). In the experiment shown in Table 1, the specific activity was estimated at about 2 x $10^9$ units/mg protein, based on an estimated protein content of <2 to 5 μg/ml in the peak column fractions. The peak fraction (1 ml) eluting at pH 5.2 from several preparative runs showed an estimated
Table 2. *Elution profiles on zinc chelate chromatography of interferon from the osteosarcoma cell line, MG-63, and from diploid fibroblasts (strain E1SM)*

<table>
<thead>
<tr>
<th>Designation of fractions</th>
<th>Volume (ml)</th>
<th>Total interferon activity from MG-63 cells Units × 10⁻⁶</th>
<th>%</th>
<th>Total interferon activity from diploid fibroblasts (E1SM) Units × 10⁻⁶</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input (CPG-purified, dialysed at pH 7.4)</td>
<td>100</td>
<td>89.12 × 10⁻⁶</td>
<td>100</td>
<td>24.2 × 10⁻⁶</td>
<td>100</td>
</tr>
<tr>
<td>Void + pH 7.4 wash</td>
<td>130</td>
<td>0.63</td>
<td>--</td>
<td>0.04</td>
<td>--</td>
</tr>
<tr>
<td>pH 5.9 wash</td>
<td>200</td>
<td>44.62 × 10⁻⁶</td>
<td>50.06</td>
<td>1.5 × 10⁻⁶</td>
<td>6.6</td>
</tr>
<tr>
<td>pH 5.2 eluate*</td>
<td>15</td>
<td>44.08 × 10⁻⁶</td>
<td>49.46</td>
<td>22.2 × 10⁻⁶</td>
<td>91.4</td>
</tr>
</tbody>
</table>

* Pool of fractions eluting between pH 5.6 and 4.2.

Table 3. *Neutralization of different fibroblast interferon fractions by antibody from a goat immunized against fibroblast interferon purified by CPG adsorption and zinc chelate chromatography (fraction eluting at pH 5.2)*

<table>
<thead>
<tr>
<th>Interferon fraction</th>
<th>Neutralization*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular origin</td>
<td>Purification schedule</td>
</tr>
<tr>
<td>E1SM</td>
<td>Crude</td>
</tr>
<tr>
<td>E1SM</td>
<td>CPG–zinc chelate, pH 5.2 fraction</td>
</tr>
<tr>
<td>E1SM†</td>
<td>CPG–zinc chelate, pH 5.2 fraction</td>
</tr>
<tr>
<td>E1SM†</td>
<td>CPG–zinc chelate, pH 5.9 fraction</td>
</tr>
<tr>
<td>MG-63</td>
<td>CPG–zinc chelate, pH 5.2 fraction</td>
</tr>
<tr>
<td>MG-63</td>
<td>CPG–zinc chelate, pH 5.9 fraction</td>
</tr>
<tr>
<td>Leukocyte PIF‡</td>
<td>&gt;1/1</td>
</tr>
</tbody>
</table>

* Dilution neutralizing 1000 units/ml.
† Beyond passage level no. 20.
‡ Gift from Dr K. Cantell, Central Public Health Laboratories, Helsinki, Finland.

Initially, the elution of a small proportion of interferon in the pH 5.9 wash was considered to be the result of non-specific leakage. However, it was found subsequently that the osteosarcoma cell line MG-63 consistently produced interferon, a high proportion of which eluted in the pH 5.9 wash fractions. Interferons from diploid fibroblasts and from MG-63 cells were therefore processed through the protocol outlined in Fig. 1, i.e. consecutive purification by CPG adsorption and by zinc chelate chromatography. On CPG both interferons behaved identically. However, on zinc chelate chromatography a salient difference became apparent, as evident from the data in Table 2. While with diploid cell interferon, only 6% of the interferon eluted in the pH 5.9 wash, this fraction amounted to 50% with MG-63 cell interferon. A similarly high proportion of interferon eluting at pH 5.9 was also consistently found in interferon produced by a diploid cell strain (E1SM) of high passage level.

On rechromatography each of the two interferon fractions eluted quantitatively in a single peak at their corresponding pH. This indicates that the peculiar elution profile was not due to non-specific factors such as accompanying impurities.
To rule out the possibility that the interferon in one of the two elution peaks was of the leukocyte type, neutralization reactions were performed using a specific anti-fibroblast interferon antibody raised against interferon eluting from the columns at pH 5.2. Table 3 shows that the antibody neutralized both the interferons eluting at pH 5.9 or 5.2, but not leukocyte interferon. Exposure for 30 min at 56 °C did not differentiate the interferons eluting at different pH, as both lost 80% of their activity.

Analysis of fibroblast interferon purified to a specific activity of $2 \times 10^9$ units/mg by CPG adsorption and zinc chelate chromatography

The results presented above suggested that the combination of CPG adsorption and zinc chelate chromatography allowed complete purification of the population of interferon molecules eluting at pH 5.2. To verify this, radiolabelling experiments were performed. Two labelling techniques were found suitable: in vivo labelling by adding radioactive amino acids during interferon synthesis by the cells, and in vitro labelling by $^{14}$C-formaldehyde.
For *in vivo* labelling, one roller bottle containing a monolayer of 50 x 10^6 E,SM fibroblasts was primed and superinduced with poly(rI).poly(rC). The routine procedure, described in detail elsewhere (Billiau et al., 1979), was used except that the medium contained only 0.75% of the normal concentration of amino acids during the induction stage and 250 μCi 14C-protein hydrolysate in 30 ml of the same medium containing 0.09 mg/ml HPPF during the production phase. The culture fluid was harvested after 20 h, centrifuged at 1000 g for 15 min and processed through the CPG–zinc chelate schedule described in Fig. 1. The fraction eluting at pH 5.2 was concentrated to 50 μl by dialysis against a viscous Ficoll solution, followed by dialysis against Dulbecco’s PBS. The sample was then analysed by discontinuous SDS–PAGE and autoradiography. Fig. 3 (b) shows the autoradiograph with only a single radioactive band of apparent mol. wt. 22000. The experiment showed only the absence of contaminating protein synthesized by the fibroblasts, and would not have detected impurities originating in the protein added to the culture (e.g. HPPF or calf serum).

For this reason an additional experiment was performed using 14C-formaldehyde labelling of the product eluting from the column. The autoradiographical profile obtained with this gel is shown in Fig. 3 (a). The most prominent band had an apparent mol. wt. of 22000 and corresponded to the antiviral activity in a parallel gel. Minor bands of protein, amounting to less than 5% of the total (as estimated by densitometry), were also visible. They could originate either in the calf serum used for cell culturing or in human plasma proteins added during production and after CPG purification. We favour the second alternative, in view of the results obtained with an antiserum raised in a goat by immunization with the CPG + zinc chelate-purified interferon. In immunodiffusion this serum reacted strongly with HPPF (threshold antigen concentration 0.005 mg/ml protein) and only weakly with bovine serum albumin (0.1 mg/ml) or fibroblast lysate (1 mg/ml). Moreover, the precipitin lines with these different antigens were cross-reactive indicating that the main antibody was directed to HPPF.

**DISCUSSION**

Human fibroblast interferon produced by diploid cells or by the osteosarcoma cell line MG-63 and prepurified by CPG adsorption, was separated into two distinct fractions by chromatography on zinc chelate columns. One fraction eluted at pH 5.2. The amount of protein under this peak was extremely small and the total quantities available did not allow for accurate determination. In fact, the protein concentration of the samples was such that a twofold difference in estimated figures was probably not meaningful (see Methods). Hence, the specific activity could only be approximately estimated at about 2 x 10^9 units/mg protein. This figure is in agreement with the theoretical estimate of Ng & Vilcek (1972) and higher than that recently reported by other researchers for HuIFN-β (Berthold et al., 1978; Knight et al., 1980) and for HuIFN-α (Rubinstein et al., 1979; Zoon et al., 1979). The purification procedures of these workers were multi-step (7 to 10) with low recoveries suggesting the possible presence of inactive interferon in the end-products, thus lowering their specific activity. The procedure employed by Knight et al. (1980) was less elaborate than that of the above workers but the final step was an SDS preparative gel electrophoresis with a relative low recovery of 5 to 20% and a range of specific activity of 2 x 10^8 to 8 x 10^8 units/mg. In addition, our estimated value appears more realistic in view of the specific activity of 2 x 10^8 units/mg of partially purified material reported by Edy et al. (1977).

That the purification achieved by the two-step CPG adsorption + zinc chelate chromatography was only near-complete was demonstrated by autoradiographical SDS–PAGE analysis of radiolabelled product. With *in vivo* labelled interferon only a 22,000 mol. wt. band, corresponding to the interferon activity, was detectable. However, with *in vitro* labelled product some minor contaminants, probably representing less than 5% of the interferon protein, could be detected. When this material was used as an antigen in a goat, a
strongly interferon-neutralizing antibody (1/200 000 to 1/400 000) was obtained, which also reacted in immunodiffusion with HPPF, suggesting that the contaminants originated in the HPPF used as a stabilizer during production and purification. This interpretation was also supported by comparing the autoradiographs with those obtained from 125I-labelled HPPF (Heine et al., 1980).

Using interferon originating from diploid cell strains, the overall recovery was about 50%. The significance of this finding is the achievement of near-complete purification of human fibroblast interferon by a simple two-step procedure with high recovery rate.

On zinc chelate chromatography part of the biological activity eluted ahead of the pH 5.2 peak fraction, i.e. during the washing of the zinc chelate column with buffer at pH 5.9. This material still contained a relatively large amount of contaminating protein. With most interferon preparations from diploid cells the pH 5-9 fraction represented less than 10% of the total eluted activity and was therefore initially disregarded as the result of non-specific leakage. However, with interferon from the osteosarcoma cell line and from some high passage diploid cell strains it represented up to 50% of the total eluted activity. On rechromatography this fraction behaved consistently. Its biological activity could however be neutralized by an antiserum raised against the highly pure interferon fraction eluting at pH 5.2. This seems to indicate that there are no major antigenic differences between the interferons eluting at pH 5.2 and pH 5.9. Electric charge heterogeneity has already been described for various interferons, using isoelectric focusing (Schonne et al., 1970; Törmä & Paucker, 1976; Morser et al., 1978). In the case of human fibroblast interferon it was demonstrated that there were three populations of molecules with different isoionic points, which could be converted into one single variant by treatment with neuraminidase (Morser et al., 1978). Treatment of MG-63 cells with the glycosylation inhibitor tunicamycin reduced the total yield of interferon by 90%, but did not alter the proportion of the two variants (data not shown), indicating that they are both glycosylated. This does not rule out the possibility of more subtle quantitative or qualitative differences in glycosylation as described by Morser et al. (1978). Several other explanations may be given for our findings. For instance, the fraction eluting at pH 5.9 might consist of fibroblast interferon molecules not basically different from those in the fraction eluting at pH 5.2, but linked to carrier protein molecules. Therefore, it would be premature to consider the interferons eluting at pH 5.2 and 5.9 as having a different molecular structure.

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