Comparison of In Vitro and In Vivo Labeling of Virus-Induced L-Cell Interferon

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ABSTRACT Preparations of NDVαv-induced L-cell interferon were labeled in vitro with $^{125}$I and $^3$H gas, or in vivo through incorporation of amino acids-$^3$H during synthesis. Prior to purification, more than 90% of the interferon titer was lost during in vitro labeling by either procedure, whereas 34% of the initial activity of in vivo-labeled material was preserved during preparatory handling. Purification by carboxymethyl-Sephadex chromatography and electrophoresis in polyacrylamide gels was about 100-fold, and electrophoretic profiles revealed close concordance between isotopes and interferon titers in all instances. Noninterferon proteins from control cells, although less extensively labeled with tritium during synthesis than proteins from interferon-producing cells and released in lesser amounts, also contained components of identical electrophoretic mobility and distribution in acrylamide gels as interferon. The highest specific activity (6 × 10$^6$ U/mg protein) but lowest cpm per interferon unit ratio (0.3) were exhibited by in vivo-labeled interferon. The advantage of better isotope incorporation through in vitro labeling techniques was largely offset by extensive losses in interferon activity.

INTRODUCTION Recent progress in the purification of L-cell interferon (1) prompted attempts to incorporate an isotopic label into its structure. The addition of tritiated amino acids to the culture medium at the time of maximal liberation of virus-induced L-cell interferon resulted also in the simultaneous release of proteins into which the isotope had become incorporated (2). In the absence of viral stimulation, both the total amount of protein liberated and the incorporation of label were less than that in comparable materials from interferon-producing cells. In subsequent steps which involved primarily chromatography on carboxymethyl-Sephadex and electrophoresis in polyacrylamide gels, the radioactive preparation was purified approximately 500-fold.
The final product which showed excellent agreement between the electrophoretic profiles of interferon and isotope was calculated to contain in the order of $1 \times 10^7$ U of interferon per mg of protein, based on elimination of labeled proteins during purification.

In view of the advisability of using highly labeled preparations in projected studies on the interaction between interferon and host cells, further efforts were directed toward obtaining improved radioactive materials by means of in vitro labeling procedures. Current methods for incorporation of $^{125}$I and $^3$H were modified to minimize inactivation of interferon, and preliminary results are described in this report.

MATERIALS AND METHODS

Cell Cultures

L cells were propagated in suspension as previously described (4, 5), except for the use of Eagle’s basal medium in spinner salt solution instead of medium CMRL-1066. For attachment to glass, Eagle’s basal medium in Hank’s solution, supplemented with 10% calf serum, 0.15% bicarbonate, and antibiotics, was employed.

Interferon was assayed on monolayers of L(MCN) cells (6) grown in: Scherer’s maintenance solution, 60%; medium 199, 30%; and inactivated horse serum, 10%.

Viruses

Newcastle disease virus (Victoria strain) was routinely passaged in chick embryos. For stimulation of interferon, virus-containing allantoic fluids were first dialyzed overnight in the cold against 20 volumes of 0.1 M phosphate-buffered saline at pH 7.2 (PBS). 15-20-ml amounts were then exposed to a 15-w germicidal lamp at a distance of 7 inches for 10-20 sec. The emission was 5000 ergs/cm$^2$ per sec. During irradiation, the preparations were rocked mechanically at the rate of 90 times per min with excursions of 1 inch. The partially ultraviolet-inactivated virus will be referred to as NDV$_{uv}$.

Vesicular stomatitis virus (VSV, Indiana strain) was carried for an undetermined number of dilute passages in L cells.

Production and In Vivo Labeling of Interferon

About $10^7$ L cells contained in 50 ml of medium were dispensed into 500-ml round prescription bottles held in a roller drum rotating at 8 revolutions per hr (7). The bottles were refed on the 3rd day and used for production of interferon 1 day later. The medium was replaced by 10 ml of undiluted NDV$_{uv}$, corresponding to 300 plaque-forming units (p.f.u.) per cell before irradiation, and the cells were incubated on the drum for 1 hr at 37°C. The cultures were then refed with 10 ml of serum-free medium and returned to the
incubator for an additional 7 hr. At that time, the medium was replaced by one of the same composition except for the substitution of protein hydrolysate-3H (Schwarz Bio Research Inc., Orangeburg, N. Y.), at a concentration of 10 μCi/ml, instead of the amino acids normally present. Interferon was harvested after a total incubation of 12-14 hr since contact with NDVv.

To obtain initial protein and radioactive readings, 5 ml of the crude interferon were dialyzed in the cold against 4 × 4 liters of 0.1 M phosphate buffer at pH 8. Interferon activity of the starting material was measured on a separate aliquot after dialysis against pH 2 (8) and subsequently against PBS. The remainder of the crude interferon was concentrated 20 times by ultrafiltration, dialyzed against 0.01 M sodium acetate buffer at pH 4.5 (9) and against four changes of 4 liters each of 0.1 M phosphate buffer at pH 6.

For measuring radioactivity, 0.1 ml samples were distributed into vials containing Bray's solution (10) and counted in a Tri-Carb liquid scintillation spectrometer (Packard Instruments Co., Downers Grove, Ill.).

Controls were obtained by substituting ultraviolet-irradiated dialyzed allantoic fluid from uninfected eggs for virus. Otherwise, the same procedure as that used for interferon was followed.

**In Vitro Labeling with 125I**

Interferon was prepared as described in the preceding paragraph, except that tritiated amino acids were omitted from the collection medium. For labeling with iodine, the method originally reported by Greenwood et al. (11) was modified as follows: to 1 ml of nonpurified interferon which had been dialyzed against borate buffer (sodium borate-HCl) at pH 8.0, 0.2 ml of 125I (1 mCi) in carrier-free solution of NaOH were added, followed by 0.1 ml of chloramine-T in borate buffer (0.7 mg/ml). The solution was mixed for 5 min when 0.1 ml of sodium metasulfoxide (Na2S2O5) in borate buffer (0.63 mg/ml) was added to reduce the excess of chloramine-T. Immediately thereafter, the material was filtered on Sephadex G-100, using 0.01 M phosphate-buffered saline as eluting buffer, to separate the above ingredients which had been shown to cause rapid inactivation of interferon. Such materials have retained full activity after storage in the cold for several months. The interferon-containing eluates were pooled, concentrated, and extensively dialyzed against 4 × 4 liters of 0.1 M phosphate buffer at pH 6.0 or until the radioactivity in the dialysate had become stationary and fallen to background levels. 125I was counted either directly in a Packard Auto-Gamma spectrometer, or in a Packard Tri-Carb scintillation counter.

**In Vitro Labeling with Tritium**

Concentrated nonpurified interferon or control materials, after exhaustive dialysis against 0.1 M phosphate buffer at pH 6.0, were shipped to the Tritium
Labeling Department, New England Nuclear Corp., Boston, Mass. for labeling with tritium gas (procedure NET-263). Handling included the following steps: (a) the refrigerated materials were reduced to powder by freeze-drying; (b) they were exposed to tritium gas (3 Ci) for 2 wk at subfreezing temperature; (c) after removal of tritium gas, the samples were dissolved in cold distilled water to a concentration of 1 mg of protein per ml; and (d) labile tritium was eliminated by extensive dialysis against phosphate buffer, as described for the iodine labeling method, and counts were obtained as mentioned previously.

**Assay of Interferon**

Serial fourfold dilutions of interferon were inoculated in 0.5 ml volumes into 30-ml tissue culture flasks (Falcon Plastics, Los Angeles, Calif.), seeded with 6 × 10^6 cells. After incubation overnight at 37°C, the cultures were drained and challenged with approximately 100 p.f.u. of VSV. 1 hr later, overlay medium was applied which consisted of a mixture in equal proportions of 2.4% Difco agar and twice the usual concentration of growth medium with neutral red 1:20,000. Plaques were scored on the 2nd or 3rd day, and the 50% plaque reduction end point was computed from a standard regression curve. One unit was defined as the highest dilution of interferon in 0.5 ml volume which reduced the number of VSV plaques found in controls by half.

**Proteins**

Determinations were made in a Beckman DB spectrophotometer according to Lowry et al. (12) using crystalline bovine plasma albumin as a standard.

**Concentration of Interferon**

Materials were syphoned into dialyzer tubing (diameter 3/32 inch, inflated; Arthur H. Thomas Co., Philadelphia, Pa.) and subjected to a vacuum of approximately 20 psi. The flow rate was 5 ml/hr, and the biologic activity of crude interferon was quantitatively accounted for after 20-fold concentration (9). Losses for chromatographed interferon were variable, but minimized by the addition of bovine plasma albumin (BPA), and adjusted to give a final concentration of 0.5% (w/v) on termination of ultrafiltration.

**Chromatography**

Interferon and controls were partially purified in a jacketed column (K25/45; Pharmacia Fine Chemicals Inc., New Market, N.J.) of carboxymethyl-Sephadex C-25 (CMS). The loading ratio was 1 mg of protein to 10 ml of gel volume. The flow rate was adjusted to approximately 60 ml/hr, and fractions were collected in a refrigerated cabinet (Beckman, model 133A). Optical density at 280 mμ and pH were monitored by continuous flow cell assemblies.
Polyacrylamide Gel Electrophoresis

Pools of interferon eluted from CMS were concentrated 10-fold by ultrafiltration in the presence of 0.05% BPA. Samples of 0.4–0.8 ml in 0.1 m phosphate buffer (pH 6–7), containing 20% sucrose, were introduced into chambers interspersed between two gels (3). Columns measuring 87 × 5 mm consisted of 7.5% gel (w/v), composed of 28% acrylamide and 0.73% methylene bisacrylamide, in acid buffer (pH 4.3). Polymerization was carried out in the presence of 0.005% riboflavin (13). A Canalco model 12 electrophoretic apparatus (Canal Industries, Bethesda, Md.) was employed, and electrophoresis proceeded in B-alanine electrode buffers at pH 4.3 by applying 5 ma per gel for 60–120 min. Methyl green at a concentration of 0.001% was added as tracking dye to the anode buffer.

Upon termination of each run, gels were sliced into 1 mm discs, and from two to three of these were pooled to form one fraction. Interferon was eluted during several days in the cold into 0.5 ml volumes of PBS containing from 0.05 to 0.5% BPA. For measurement of radioactivity, 0.1 ml portions were placed into vials containing Bray's solution.

Electrofocusing

The method developed by Vesterberg and Svensson (14) was employed. An LKB 8101, 110 ml ampholine electrofocusing column was filled with 24 fractions, each consisting of 4.6 ml of carrier ampholytes covering a pH range of 3–10, dissolved in sucrose solutions of different densities varying from 50 to 1%. Interferon samples were incorporated into several of the fractions in the middle portion of the column. A current of 600 v was applied for 48 hr. Upon termination of the run, 3-ml fractions were collected, bovine plasma albumin (BPA) at a final concentration of 0.5% was added, and pH, radioactivity, and interferon determinations were carried out in the usual manner.

Sterilization of Interferon Samples

Nonsterile materials were irradiated for 2 min by ultraviolet light as described for inactivation of NDV. Interferon titers remained unaffected by this procedure.

RESULTS

Purification and Electrophoresis of Interferon Labeled In Vitro with $^{125}$I

In preliminary experiments, iodine in combination with and without the ingredients used in the coupling procedure was examined for any direct inactivating effect on interferon. Crude concentrated interferon preparations were subdivided into portions exposed either to $^{125}$I alone, to Chloramine-T mixed with Na$_2$S$_2$O$_5$, or to all three compounds simultaneously under the
exact conditions employed in the labeling technique. Immediately following treatment, the materials were titrated for residual biologic activity and compared with controls which had not sustained contact with any of the above agents. The results of two separate experiments presented in Table I show that treatment of interferon with a mixture of chloramine-T and Na$_2$S$_2$O$_5$ caused an approximately five-fold reduction in titer, whereas all three ingredients together occasioned 8–20-fold losses in activity. $^{125}$I alone had apparently no direct inactivating influence on interferon.

As a result of these findings, it was decided to filter the iodinated interferon preparation immediately after completion of the labeling procedure through Sephadex G-100, in order to provide a rapid and satisfactory separation of the injurious components from the labeled interferon material. The appropriate fractions were then pooled, concentrated by ultrafiltration to a convenient volume, and subjected to chromatography on carboxymethyl-Sepha-

| TABLE I
| INACTIVATION OF INTERFERON BY CHLORAMINE-T AND SODIUM METASULFOXIDE |
| Treatment of interferon$^*$ |
| Expt. No. | None | $^{125}$I | Chloramine-T + Na$_2$S$_2$O$_5$ | Na$_2$S$_2$O$_5$ |
| Units | % | Units | % | Units | % | Units | % |
| 1 | 15,000 | 100 | 30 | 20 | 1710 | 11.5 |
| 2 | 51,200 | 100 | 51,200 | 100 | 2400 | 4.6 |

$^*$ 5 min at room temperature.

dex C-25 (CMS), as described. Interferon-containing eluates were again pooled, and after admixture of BPA (0.05%) they were concentrated by the same method. This material was subsequently examined by electrophoresis in polyacrylamide gels.

An analysis of the fates of interferon, radioactivity (cpm), and proteins during the various steps of this procedure is given in Table II. A major reduction in interferon activity (95%) occurred during the iodination step and preparatory handling for chromatography. At the same time, protein was lost to the extent of 79%. During chromatography, taking the pre-CMS values as a starting point, more than half (56%) of the input interferon activity was recovered, as contrasted with 2.14% for $^{125}$I and less than 1.4% for protein. Following electrophoresis, an additional two- to fourfold dissociation from interferon of $^{125}$I-labeled proteins took place, depending on whether total or peak eluates are considered, whereas interferon was quantitatively accounted for. Radioactivity and interferon profiles obtained in polyacrylamide gel are presented in Fig. 1. Interferon activity was distributed over
TABLE II

Purification of NDVuv-induced L-cell interferon after in vitro labeling with \(^{125}\)I

| Purification step                        | Interferon | Radioactivity | Protein |
|-----------------------------------------|------------|---------------|---------|
|                                         | Interferon | Radioactivity | Protein |
|                                         | Unit(s)    | cpm           | mg      | cpm/ifu |
| Nonpurified                             | 4.10 \(\times\) 10^6 | 100           | —       | 29.6   | 100 | —     |
| Pre-CM-Sephadex                        | 2.05 \(\times\) 10^4 | 5             | 2.50 \(\times\) 10^6 | 100 | 6.3 | 21.2 | 121 |
| CM-Sephadex                            | 1.18 \(\times\) 10^4 | 2.8           | 5.37 \(\times\) 10^4 | 2.14 | <0.1 | <0.3 | 4    |
| Polyacrylamide gel electrophoresis      |             |               |         |         |     |      |       |
| Total                                   | 1.56 \(\times\) 10^4 | 3.8           | 2.52 \(\times\) 10^6 | 1.0  | —   | —   | 1    |
| Peak                                    | 1.04 \(\times\) 10^4 | 2.5           | 1.29 \(\times\) 10^4 | 0.51 | —   | —   | 1    |

* Interferon units determined by plaque-inhibition assay with VSV.
† Counts per minute.
§ Interferon-associated cpm.

![Migration and distribution in polyacrylamide gels of purified NDVuv-induced L-cell interferon labeled in vitro with \(^{125}\)I.](image)

Figure 1. Migration and distribution in polyacrylamide gels of purified NDVuv-induced L-cell interferon labeled in vitro with \(^{125}\)I.

Purification, Electrophoresis, and Electrofocusing of Interferon Labeled In Vitro with Tritium

An interferon preparation which had been exposed to tritium gas was subjected to the various purification steps indicated in Table III. As with the iodinated preparation analyzed in Table II, the major loss in interferon titer occurred in the course of the labeling procedure (92%). Proteins were diminished to a lesser degree. Recovery of interferon after CM-Sephadex was
25% (or 2% of the initial titer), as against 5.4% for tritium and less than 3.1% for proteins. The best separation was obtained by electrophoresis in polyacrylamide gels which permitted more than quantitative recovery of interferon and the removal of 94% of nonassociated label. A representative profile of the distribution of tritium and interferon elutable from the gels is illustrated in Fig. 2. The two major peaks of interferon are closely contoured by the radioactive label, while the bulk of the isotope, together with proteins of a more alkaline nature, had migrated ahead of the interferon into the electrode buffer system (3).

In an effort to see whether, on the basis of molecular charge alone, a further dissociation between isotopic label and interferon activity was possible,

### TABLE III

| Purification step                  | Interferon | Radioactivity | Protein |
|-----------------------------------|------------|---------------|---------|
|                                   | ifu*       | %             | cpm†    | mg     | %         | cpm/ifu |
| Nonpurified                       | 1.15 × 10⁶ | 100           | 33.1    | 100    | —         | —       |
| Pre-CM-Sephadex                   | 9.24 × 10⁴ | 8             | 7.00 × 10⁸| 100    | 13.0      | 39      | 7575    |
| CM-Sephadex                       | 2.30 × 10⁵ | 2             | 3.79 × 10⁸| <0.4   | <1.2      | 1644    |
| Polyacrylamide gel electrophoresis| Total      | 2.96 × 10⁴    | 2.5     | 2.35 × 10⁸| 0.33      | —       | 79      |
|                                  | Peaks      | 2.51 × 10⁴    | 2.2     | 1.89 × 10⁸| 0.27      | —       | 75      |
|                                  | Electrofocusing | 1.23 × 10⁵  | 1.06    | 1.05 × 10⁸| 0.15      | —       | 90      |

* Interferon units determined by plaque-inhibition assay with VSV.
† Counts per minute.
§ Interferon-associated cpm.

the two dominant interferon peaks, representing 84% of the interferon eluted from polyacrylamide gels and 80% of the elutable isotope, were pooled and subjected to isoelectric focusing. The results illustrated in Fig. 3 show that two major radioactive peaks had formed in the pH gradient, which were situated at pH 5.2 and 9.3, respectively, and a minor ³H peak was found at pH 7.1. The bulk of interferon activity lay between pH 6 and 9.5 and was thus largely absent in the pH 5.2 region. The evaluation presented in Table III indicates that 44.5% of the radioactive counts measured at the start of the electrofocusing procedure had been distinctly separated from the region where interferon was detectable. On the other hand, interferon was inactivated to the extent of 51% during handling, so that losses in cpm and titer were almost parallel. Unless one assumed that interferon was present in the ³H peak at pH 5.2 and was preferentially inactivated in that region, one may tentatively conclude that further dissociation had in fact occurred.
Figure 2. Migration and distribution in polyacrylamide gels of purified NDV<sub>uv</sub>-induced L-cell interferon labeled in vitro with tritium gas.

Figure 3. Distribution by electrophoresing of isotope and biologic activity of in vitro <sup>3</sup>H-labeled purified NDV<sub>uv</sub>-induced L-cell interferon after electrophoresis in polyacrylamide gels.
Purification and Electrophoresis of Interferon Labeled In Vivo with Tritiated Amino Acids

The preceding in vitro-labeled preparations were then compared with an interferon which had been labeled in vivo, as detailed under Materials and Methods. This interferon was then handled exactly as those preparations subjected to in vitro labeling with iodine and tritium. A survey of interferon, cpm, and protein determinations during the various steps of the purification procedure is shown in Table IV. In this case, the excessive loss in titer encountered during in vitro labeling could be avoided. Nearly 40% of the pre-CMS titer but only 12% of the cpm harbored by the same material were recovered after CMS chromatography. Electrophoresis in polyacrylamide gels permitted again greater than quantitative recovery of biologic activity, whereas 82% of the input label was eliminated. The distribution of the 18% of cpm which eluted from the gels together with interferon is presented in Fig. 4. Approximately one-third of the tritium counts closely outlined the steep interferon profile, whereas the largest portion of the remainder migrated more slowly and was essentially devoid of interferon activity.

Table V summarizes the specific activities and extent of purification achieved with all three types of labeled interferon. The improvement in specific activities of the tritiated materials after chromatography was less than optimal (three- to fivefold), whereas iodinated interferon was purified approximately 30 times, which reflects more accurately the effectiveness with which extraneous proteins can be separated from interferon during this step. In the course of polyacrylamide gel electrophoresis, purification was
PURIFICATION AND MECHANISM OF ACTION

Figure 4. Migration and distribution in polyacrylamide gels of purified NDV<sub>vv</sub>-induced L-cell interferon labeled in vivo with amino acids-<sup>3</sup>H (15).

Table V
CALCULATED SPECIFIC ACTIVITIES ON PURIFICATION OF IN VITRO AND IN VIVO LABELED INTERFERONS

| Labeling procedure          | In vitro | In vivo AA-<sup>3</sup>H* |
|-----------------------------|----------|---------------------------|
|                             | Spec. A.1| Pur.§ | Spec. A. | Pur. | Spec. A. | Pur. |
| Pre-CM-Sephadex             | 3.25 X 10<sup>3</sup> | 1X | 7.10 X 10<sup>3</sup> | 1X | 7.51 X 10<sup>4</sup> | 1X |
| CM-Sephadex                 | 9.81 X 10<sup>4</sup> | 30.2X | 3.26 X 10<sup>4</sup> | 4.6X | 2.35 X 10<sup>5</sup> | 3.4X |
| Polyacrylamide gel electrophoresis peak | 3.93 X 10<sup>4</sup> | 121X | 7.17 X 10<sup>3</sup> | 101X | 6.0 X 10<sup>4</sup> | 80X |

* Tritiated amino acids.
† Specific activity (U/mg of protein), calculated from cpm/ifu ratios of Tables II, III, and IV.
§ Purification as compared to initial material.

variable, ranging from 4- to 20-fold so that ultimately all materials had been purified to about the same degree, namely from 80 to 120 times. However, the quality of the in vivo-labeled tritiated interferon, because inactivation could be minimized during handling, was from 8 to 15 times superior to that of the in vitro-labeled preparations.

Electrophoresis of Control Cell Proteins Labeled In Vitro and In Vivo with Tritium

In order to compare the interferons labeled by three different methods with controls obtained from cells which had not been exposed to NDV<sub>vv</sub>, two
additional materials were obtained as follows. First, medium was collected from cultures treated with normal irradiated allantoic fluid, concentrated, and then subjected to tritium gas labeling as described. Second, cultures were exposed to tritiated amino acids under conditions identical with those used for in vivo labeling of interferon, except that the viral step was omitted. Both materials, which did not harbor detectable antiviral activity, were subsequently purified in the same manner as indicated for interferons. Following chromatography on CM-Sephadex, the main difference between the two controls lay in the cpm per protein ratios. The in vivo-labeled material contained only about \( \frac{1}{10} \) the radioactivity per protein equivalent exhibited by the corresponding interferon, whereas the degree of isotope incorporation was similar for the in vitro-labeled control and interferon preparations. For electrophoresis in polyacrylamide gel, chromatographed nonlabeled interferon was admixed to the controls to see whether the peak biologic activities were at variance with or corresponded to the migration profiles in the same gels of proteins liberated from nonstimulated cells. Fig. 5 depicts the results obtained with the in vivo-labeled control. The radioactive distribution of control proteins occupies a broader area of the gel than the corresponding interferon group shown in Fig. 4. Moreover, a distinct peak was noticed which coincided with the major zone of activity in the admixed interferon. Therefore, it is apparent that proteins liberated from nonstimulated cells in smaller amounts and less extensively labeled than those released from stimulated cultures, contain after approximately 100-fold purification components of strikingly similar physical properties as interferon proteins. Comparable results were obtained with in vitro-labeled control proteins as illustrated in Fig. 6. Because of the indiscriminate incorporation of tritium under these conditions, the isotope profile reveals considerable breadth, but it appears that a major radioactive peak corresponds to the migration profile of the added nonlabeled interferon.

![Figure 5. Migration and distribution in polyacrylamide gels of purified L-cell control proteins labeled in vivo with amino acids-\(^{3}H\) and mixed with purified, nonlabeled NDVuv-induced L-cell interferon.](image-url)
DISCUSSION

Previous studies have shown that a tritium label could be incorporated into proteins during in vivo synthesis of interferon (2). Following purification by chromatography and electrophoresis in polyacrylamide gels, a small portion of the isotope was not separable from interferon activity (3). In the present report, the possibility was explored whether by means of established methods for in vitro labeling of proteins, the intensity of the label as well as the efficiency of incorporation of isotope may be raised. For the first purpose $^{125}$I was selected because of its long half-life and as a good source for high energy radiation, commonly used for labeling of proteins (11). For the second, tritium gas was chosen because the prolonged exposure to the isotope permits a greater exchange of hydrogen atoms. In addition, the method provides an opportunity to compare interferons of identical origin, collected during the same time interval, labeled by two different methods with the same source of radiation.

The data disclosed that in vitro labeling by either of the methods used was markedly injurious to the biologic activity of interferons. However, the advantage of the in vitro processes resided in the higher cpm:ifu ratios which could be achieved during the initial labeling step.

The differences in purification of the three preparations during chromatography on CM-Sephadex are not considered significant. Purification of the tritiated materials during this step was less than usually encountered, but the values obtained with $^{125}$I interferon were in line with those previously reported (15). The most significant dissociation between interferon and isotopically-labeled proteins occurred in the course of electrophoresis in...
polyacrylamide gels (3). From 77 to 95% of isotopes could be eliminated during this step alone without concomitant loss in interferon titer. In fact, recovery of interferon was consistently greater than quantitative (16) indicating that L-cell interferon, like rabbit interferon (E. Schonne. Personal communication) may exist to some extent in the form of aggregates which decomplex during electrophoresis.

While the electrophoretic profile of interferon usually reveals one distinct major peak, a second zone of minor activity is on occasion encountered, as in the case of the ³H gas-labeled material of Fig. 2. The presence or absence of a second, more slowly migrating peak might best be attributed to variable inactivation of interferons in the course of handling. This observation is not inconsistent with the finding that L interferon, like chick interferon, displays considerable heterogeneity with respect to electric charge (Fig. 3) (17). Slight differences were seen in the electrophoretic patterns of the more slowly moving (acidic) proteins of the ³H-labeled interferons. These may only be quantitative, but the possibility cannot be discounted that the degree of labeling of interferon-associated proteins may differ under in vitro and in vivo conditions.

In essence, the in vitro and in vivo labeling methods gave comparable results. However, the advantages of a more efficient tritium or a more intense iodine label introduced in vitro were largely offset by the extensive inactivation of interferon during handling.

Of considerable interest was the finding that control proteins, labeled by two different techniques, contained after extensive purification components which on electrophoresis in polyacrylamide gels migrated inseparably from the major area of interferon activity. Although there are indications that control cell proteins labeled in vivo with tritium vary both in quantity and in the degree of isotope incorporation from those liberated by interferon-producing cells (15), the data suggest that the methods employed may not have gone far enough to detect structural differences among interferon and control proteins. Alternatively, one could speculate that cells produce normally, and without apparent provocation, inactive but potential interferon proteins which can be activated and released on appropriate stimulation.

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