¼-inch or ½-inch point lattice placed over the photographic print. Cell profiles (50 to 100) were counted for each experimental or culture condition being evaluated. The percentage of the cell occupied by inclusion material was estimated by calculating the ratio of points falling on inclusion body material to total points falling on the bacterial cells.
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9. For morphological evaluation, the cells were fixed by adding glutaraldehyde [2.5 percent final (by volume) concentration] to the broth samples as soon as they were taken out of the fermentor. The cells were then postfixed in buffered osmium tetroxide and dehydrated in a graded acetone series. Samples for transmission electron microscopy were embedded in an epoxy resin, sectioned, and examined either unstained or stained with uranyl acetate or lead citrate (or both) with a Philips EM 400 electron microscope. Samples for scanning electron microscopy were critical point dried, Au/Pd coated by evaporation, and examined on an ETEC Autoscan electron microscope.
10. We thank J. R. Herring and W. Jones for electron microscopy assistance, D. B. Gant for technical support, and M. L. Layne and S. J. Pike for assistance.

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Crystallization of Recombinant Human Leukocyte Interferon A

Abstract. *Crystals of recombinant human leukocyte interferon A (IFLrA) were prepared, and their composition was characterized. These studies should provide the foundation for determination of the tertiary structure of IFLrA by x-ray crystallography.*

The interferons comprise a family of proteins that activate an antiviral response in animal cells. Apart from their potential use as therapeutic agents, these proteins are of interest because of their multiple effects on cells. Progress has been made in determining the primary structure of both human leukocyte (*1–5*) and fibroblast (*6–9*) interferons despite the limited amounts available. Studies on secondary and tertiary structure have been limited because of the lack of sufficient quantities of pure interferons. Through recombinant DNA technology, moderate amounts of material showing high interferon activity are now available (*10–12*).

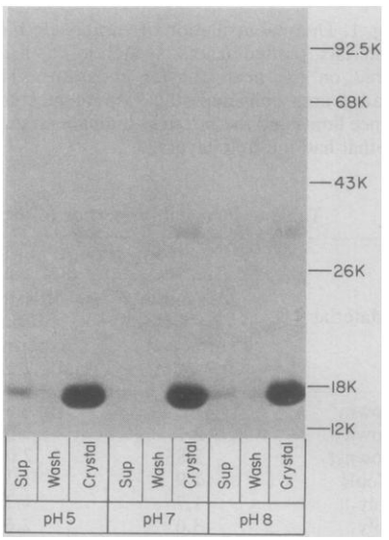
Little is known about the fidelity of transcription and translation of mammalian genes in bacteria, or about post-translational modifications that may occur. It is not certain that bacteria faithfully transcribe and translate all mammalian sequences, nor is it known whether the proteins are substantially modified. Purification of recombinant human leukocyte interferon A revealed that it is homogeneous by electrophoresis and partial amino acid sequencing (*12*); however, a substantial sequence heterogeneity might go undetected. Furthermore, heterogeneity in secondary and tertiary structure remained a real possibility.

The ability of a substance to crystallize in a uniform habit satisfies one of the classical criteria of homogeneity. Crystallization may also permit the removal of trace impurities. Furthermore, when large-ordered single crystals can be obtained, the molecule's tertiary structure may be determined by x-ray crystallography.

Numerous techniques have been developed for the crystallization of proteins (*13*); however, no generalized procedure has been discovered, and many proteins remain uncrystallized. The most widely used approach involves the addition to the protein solution of a crystallizing agent, which is commonly a salt, such as ammonium sulfate or ammonium citrate, or an organic solvent, such as ethanol or 2-methyl-2,4-pentanediol.

One of the most versatile crystallizing agents is polyethylene glycol (PEG), which combines some of the characteristics of the salts and the organic solvents (*14, 15*). Recombinant human leukocyte interferon A (IFLrA) crystallizes readily from PEG, occasionally in large crystal masses.

At low concentrations of IFLrA (0.3 to 1 mg/ml) and high concentrations of PEG-4000 (50 mg/ml) acicular crystals commonly appeared in 1 to 3 days at 4°C (Fig. 1). These crystals were centrifuged, washed with 10 percent PEG-4000, and



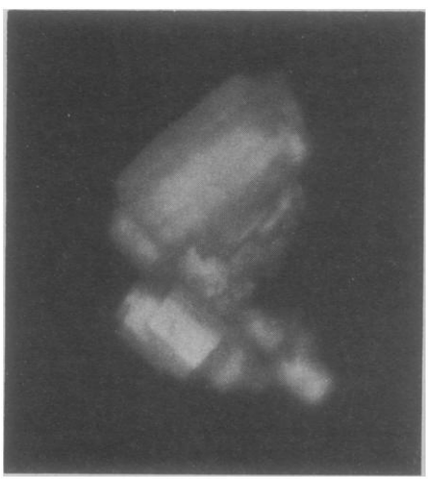


Fig. 1 (left). Crystals of IFLrA, acicular form. IFLrA prepared as previously described was dialyzed against 10 m*M* Hepes (*p*H 7.1) buffer (ammonium salt) and concentrated to 9 mg/ml by centrifugal evaporation. A 20-μl droplet containing IFLrA (2 mg/ml), NH_4-Hepes (50 m*M*, *p*H 7.1), PEG-4000 (30 mg/ml), and NaN_3 (0.5 mg/ml) was placed in the well of a siliconized spot plate and allowed to equilibrate at 4°C by vapor diffusion with PEG-4000 (50 mg/ml). Crystals appeared within 24 hours. The length of the white strip represents 0.1 mm. Fig. 2 (middle). Electrophoretic analysis of crystalline IFLrA. Crystals from a mixture similar to that described in the legend to Fig. 1 were transferred to a 1.5-ml centrifuge tube and washed twice with 100-μl portions of PEG-4000 (100 mg/ml). The crystals were dissolved in 50 μl of H_2O and subjected to polyacrylamide gel electrophoresis. Fig. 3 (right). Crystals of IFLrA, prismatic form. A droplet containing 20 μl of IFLrA (5 mg/ml), NH_4-Hepes (50 m*M*, *p*H 7.1), PEG-4000 (20 mg/ml), and NaN_3 (0.5 mg/ml) was placed in a well of a siliconized spot plate. An amorphous precipitate formed immediately, from which the crystals grew during the following week.

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analyzed by gel electrophoresis (Fig. 2). On the basis of its electrophoretic mobility, the crystalline material was judged identical to uncrystallized IFLrA. Bioassays showed that the crystals contained interferon activity. Furthermore, in the presence of PEG-4000 (100 mg/ml) virtually all of the active IFLrA added to the solution could be recovered in the crystals.

At high IFLrA concentrations (4 to 5 mg/ml) and low PEG-4000 concentrations (1.0 to 2.0 mg/ml), masses of large prismatic crystals appeared after about 5 days (Fig. 3).

We conclude that interferon synthesized by bacteria is sufficiently homogeneous to crystallize quantitatively. Crystals large enough for an x-ray crystallographic structure determination can be obtained. Whether these crystals give suitable x-ray diffraction patterns has not yet been demonstrated.

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Thermal Insulating Capabilities of Outdoor Clothing Materials

Abstract. *A single heat transfer measurement technique was used to determine the thermal insulating capabilities of four materials used in outdoor clothing—goose down, wool, polyester, and polyolefin. It was found that all provide very similar degrees of insulation.*

The increased interest in outdoor winter activities has produced a striking increase in the market for insulated outerwear. Although the down of northern geese has long been the filler material of choice, the growing market and the rising price of goose down have stimulated industrial chemists to search for synthetic substitutes for natural fibers. Periodically, new materials are put on the market which are claimed to have thermal insulating capabilities superior to those of goose down.

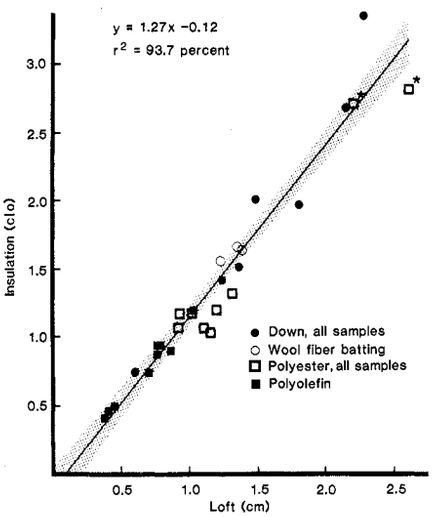

Fig. 1. Thermal insulation of various clothing materials, plotted against swatch loft as measured on the heat transfer apparatus. The shaded area delineates the 95 percent confidence limits and the asterisks indicate swatches that had multiple layers.

Using a modified guarded hot-plate technique, we measured the thermal insulation of goose down, wool, and polyester and polyolefin fibers. Our results show no superiority of any of the synthetic materials over the natural fibers as thermal insulators, although down has a marked advantage in weight per unit loft (thickness).

We prepared the materials as they would be prepared for use in the manufacture of jackets, vests, and other outerwear garments. The filler material was encased in a nylon shell. The shell was quilted with 13-cm squares and its edges were bound to form a swatch with an area of about 1 m^2. Initial loft was determined by making 12 measurements at planned points at a pressure of 0.7 g/cm^2. The weight of the swatch was determined to within 0.1 g on a top-loading balance (Mettler P2010). "Density" was calculated from measured area, loft, and weight.

Each swatch was placed horizontally over a 50 by 50 cm constant heat source. A heat sink in the form of a copper plate equal in area to the heat source was placed on top of the material. The heat sink produced a pressure of 1.2 g/cm^2; each material was examined at the same force of compression. The temperature gradient across the swatch was measured by a thermistor and the rate of heat

Table 1. Physical properties of thermal insulating materials used in clothing.

Material	Loft (cm)		Density (g/m^2 per centimeter)	Insulation	
	Measured on flat surface at 0.7 g/cm^2	Measured during experiment at 1.2 g/cm^2		clo	clo/cm
$Down_1$*	1.2	0.6	179	0.73	1.22
$Down_2$†	2.1	1.5	170	1.64	1.09
$Down_3$‡	2.8	2.0	179	2.68	1.34
Wool§	1.9	1.3	271	1.52	1.17
$Poly_1$‖	1.7	1.2	229	1.20	1.00
$Poly_1$	3.6	2.6	229	2.82	1.03
$Poly_2$¶	1.7	1.2	224	1.11	0.93
$Poly_2$	3.4	2.2	224	2.71	1.23
$Poly_3$**	1.1	0.9	368	1.02	1.13
Polyolefin	0.6	0.4	424	0.45	1.13
Polyolefin	1.0	0.8	415	0.83	1.04
Polyolefin	1.2	1.0	310	1.14	1.14

*Prime 80/20 goose down (80 percent down, 20 percent feathers) at 76.4 g/m^2. †Prime 80/20 goose down at 152.8 g/m^2. ‡Prime 80/20 goose down at 229.2 g/m^2. §Wool fiber batting. ‖One hundred percent polyester staple (Dacron II). ¶One hundred percent polyester continuous filament fiber. **One hundred percent polyester staple (Dacron 66).

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