al) sense it is designated as u. Mmm4Hbβ is accordingly u, as are Hs51.1 and Mmm117Hbα.

11. DNA from Hs51.1 was first digested with Eco RI, and the 5′ ends were labeled with ^{32}P (*12*). The end-labeled DNA was further digested with Hha I to give two Eco RI-Hha I singly labeled fragments of 170 and 350 base pairs and a doubly labeled Eco RI fragment of 465 base pairs (see Fig. 5). These three fragments were purified by polyacrylamide gel electrophoresis, and the doubly labeled fragment was redigested with Hinf I to yield two singly labeled pieces of 310 and 144 base pairs. All four fragments were partially sequenced (*12*). The experiment was repeated with Hae III as the second enzyme, which yielded four singly labeled Eco RI-Hae III fragments of 805, 109, 280, and 160 base pairs.
12. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
13. L. T. Hunt and M. O. Dayhoff, in *Atlas of Protein Sequence and Structure*, M. O. Dayhoff, Ed. (National Biomedical Research Foundation, Washington, D.C., 1976), vol. 5, suppl. 2, pp. 191-223.
14. Data made available to us by S. M. Weissman, B. G. Forget, and R. Cavellesco prior to its publication.
15. C. A. Morotta, J. J. Wilson, B. G. Forget, S. M. Weissman, *J. Biol. Chem.* **252**, 5040 (1977); F. E. Baralle, *Cell* **12**, 1085 (1977).
16. R. M. Lawn, E. F. Fritsch, R. C. Parker, G. Blake, T. Maniatis, *Cell*, in press.
17. Reviewed by B. Williamson, *Nature (London)* **270**, 295 (1977).
18. S. M. Tilghman, D. C. Tiemeier, J. G. Seidman, B. M. Peterlin, M. Sullivan, J. V. Maizel, P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 725 (1978); D. Konkel, S. M. Tilghman, P. Leder, *Cell*, in press.
19. R. W. Davis, M. Simon, N. Davidson, in *Methods in Enzymology 21D*, L. Grossman and K. Moldave, Eds. (Academic Press, New York, 1971), pp. 413-428.
20. A complete restriction enzyme map of the EK2 vector Charon 3A has been compiled with restriction site coordinates in base pairs starting at the left end of the phage (J. R. deWet, D. L. Daniels, J. L. Schroeder, B. G. Williams, K. Denniston-Thompson, D. D. Moore, F. R. Blattner, in preparation). The length of the left arm, up to the first Eco RI site, is 19,801 base pairs; the length of the right arm, from the second Eco RI site at 26,693, is 21,600 base pairs. Other Charon 3A restriction sites referred to in this report are a Kpn I site at 18,812, a Hind III site at 28,023, and a Bam HI site at 28,623.
21. Partial sequences from the Eco RI ends of the 465 base pair fragment were determined. The six amino acid sequences that could be translated from this DNA show no relationship to human β-type globin sequences. From the Eco RI sites the two ends have the provisional sequences:

5′ GAATTCTCCTTTTGTAAAAATGGGA-
5′ GAATTCTCAATGAGGATGGCAGCCC-

CCTTTATTTTTGTTTTGACCA-
TACCTGGACTTCAAGCCTGAA-

CAAAGTGAACATGGTCAGGCG 3′
GACCAGCCCCATTTTGACATC 3′

The 465-base-pair fragment does not hybridize to globin cDNA.

22. W. A. Schroeder, T. H. J. Huisman, J. R. Shelton, J. B. Shelton, E. F. Kleihauer, A. M. Dozy, B. Robberson, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 537 (1968).
23. J. Van den Berg, A. van Ooyen, N. Mantei, A. Chamböck, G. Grosveld, R. A. Flavell, C. Weissmann, *Nature (London)* **276**, 37 (1978).
24. Dr. W. M. Fitch compared available globin gene sequences by computer programs designed to detect sequence homology and to detect possible secondary structures that might be formed from the sequence.
25. The cloning experiments were done under NIH guidelines using P3 physical containment and an EK2 host vector system. We thank N. Borenstein for help and supervision of the P3 facility; J. Devereux, E. Kopetsky, D. Kiefer, J. Kucera, and T. Szeto for technical assistance; A. Bank and J. Ross for gifts of mRNA, A. Biro and R. Cavellesco for help and advice in the restriction mapping of Hs51.1; and S. Weissman, P. Leder, and T. Maniatis for making DNA sequence data available to us prior to their publication. This is paper No. 2299 from the Laboratory of Genetics, University of Wisconsin-Madison, and paper 9 in the series "Charon Phages for DNA Cloning." Supported by NIH grants AM 20120, GM 20069 (O.S.), GM 21812 (F.R.B.), CA 69075 (K.D.T.), GM 06526 (P.W.T.), GM 07133 (A.E.B. and J.E.R.), and GM 07131 (N.N.).

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Human Leukocyte Interferon Purified to Homogeneity

Abstract. *One of the species of human interferon produced by incubation of leukocytes with Newcastle disease virus was purified to homogeneity. It exhibited one peak of activity coinciding with a single protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis.*

The purification of interferon has been a continuous challenge since its discovery by Isaacs and Lindenmann (*1*) and Nagano and Kojima (*2*). Numerous attempts have been made to purify it by conventional techniques (*3-6*) as well as by affinity chromatography with antibodies (*5-7*) and other methods (*6, 8*). So far, it has been reported that human fibroblast interferon (*4*) and mouse L-cell interferon were purified to homogeneity, but no characterization of these molecules has yet been provided. Although much information is available on the physical and chemical properties of interferons as estimated by effects on antiviral activity, such indirect results are inherently only approximate. Complete physical and chemical characterization of the molecules depend on obtaining homogeneous interferon in sufficient amounts for characterization.

We have purified one species of human leukocyte interferon to homogeneity in amounts sufficient for physical and chemical characterization. Details of the purification and initial characterization, including amino acid analysis, of the homogeneous species have been described (*9*).

Leukocytes were isolated from blood of normal donors and incubated with Newcastle disease virus in a serum-free medium supplemented with casein (*10*). Interferon titers of 5000 to 20,000 U/ml were obtained. The purification procedure consisted of selective precipitations, gel filtration in 4*M* urea, and several steps of high performance liquid partition chromatography (*9*). Several species of human leukocyte interferon activity were obtained. One of the major species was purified 80,000-fold to a specific activity of 4×10^8 U/mg with bovine serum albumin as a standard (*9*).

A sample of the homogeneous interferon, treated with 2-mercaptoethanol and sodium dodecyl sulfate, was analyzed by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (Fig. 1). A single protein band of molecular weight 17,500 was obtained upon staining with Coomassie blue. After being stained, the gel was cut into 1-mm slices, each slice was homogenized in 0.4 ml of a solution containing 0.5*M* $NaHCO_3$ and 0.1 percent sodium dodecyl sulfate, and each fraction was assayed for interferon activity. A single peak of activity coinciding with the protein band was obtained.

In addition, a duplicate of the stained band was excised from the polyacrylamide gel and subjected to hydrolysis. Amino acid analysis was performed with the fluorescamine analyzer (*11*). The

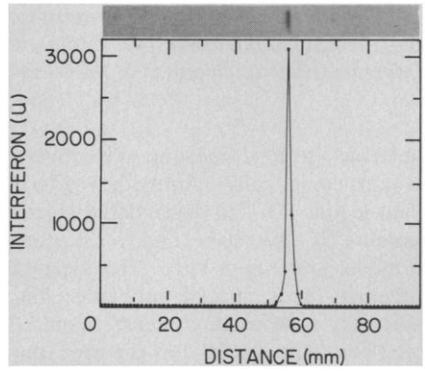

Fig. 1. Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis of human leukocyte interferon. Two samples (150,000 units each) were dissolved in 7 μl of a solution containing 1 percent (weight to volume) sodium dodecyl sulfate and 2 percent (by volume) 2-mercaptoethanol. After 60 minutes at 25°C, 7 μl of a solution containing 15.6 m*M* tris-HCl (*p*H 6.8), 40 percent (weight to volume) sucrose, and 0.1 percent (weight to volume) bromphenol blue was then added and the samples were applied to a 12.5 percent polyacrylamide slab gel in a tris-glycine (*p*H 8.4) buffer containing 0.1 percent sodium dodecyl sulfate (*12*). After electrophoresis and staining with Coomassie blue, one strip of the polyacrylamide gel was cut into 1-mm slices. Each slice was homogenized in 0.5 ml of a solution of 0.5*M* $NaHCO_3$ and 0.1 percent sodium dodecyl sulfate. Gel fragments were removed by centrifugation at low speed, and the supernatant from each homogenate was assayed for interferon activity (*9*). Interferon titers on the ordinate are expressed in terms of reference units per milliliter, calibrated against the reference standard for human leukocyte interferon (G-023-901-527) (*13*). Low recovery of interferon activity was expected after treatment of human leukocyte interferon with 2-mercaptoethanol and sodium dodecyl sulfate (*14*). The total activity recovered was 4 percent of the activity applied to the gel. The duplicate interferon band (not shown) was used for amino acid analysis and measurement of protein recovery (20 percent). Thus, 80 percent of the interferon protein was lost during electrophoresis or during staining and destaining of the polyacrylamide gel. When adjusted for this factor, the interferon activity recovered represents 20 percent of the material retained in the gel after staining and fixation.

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amino acid analysis of the interferon in the gel band was virtually identical to that obtained directly on the purified sample of human leukocyte interferon (*9*) that was applied to the gel.

We conclude that we have purified one species of human leukocyte interferon to homogeneity. The precise relation of this species to the others, to the secreted material, and to the primary gene product requires further study.

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References and Notes

1. A. Isaacs and J. Lindenmann, *Proc. R. Soc. London Ser. B* **147**, 258 (1957); ——, R. C. Valentine, *ibid.*, p. 268.
2. Y. Nagano, and Y. Kojima, *C. R. Seances Soc. Biol.* **152**, 1627 (1958).
3. E. Knight, Jr., *J. Biol. Chem.* **250**, 4139 (1975); Y. Yamamoto and Y. Kawade, *J. Gen. Virol.* **33**, 225 (1976); M. Kawakita, B. Cabrer, H. Taira, M. Rebello, E. Slattery, H. Weideli, P. Lengyel, *J. Biol. Chem.* **253**, 598 (1978).
4. E. Knight, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **73**, 520 (1976); W. Berthold, C. Tan, Y. H. Tan, *J. Biol. Chem.* **253**, 5206 (1978).
5. E. T. Törmä and K. Paucker, *J. Biol. Chem.* **251**, 4810 (1976); P. J. Bridgen, C. B. Anfinsen, L. Corley, S. Bose, K. C. Zoon, U. Th. Rüegg, C. E. Buckler, *ibid.* **252**, 6585 (1977).
6. J. De Maeyer-Guignard, M. G. Tovey, I. Gresser, E. De Maeyer, *Nature (London)* **271**, 622 (1978).
7. C. B. Anfinsen, S. Bose, L. Corley, D. Gurari-Rotman, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3139 (1974); K. Berg, C. A. Ogburn, K. Paucker, K. E. Mogensen, K. Cantell, *J. Immunol.* **114**, 640 (1975).
8. S. Bose, D. Gurari-Rotman, U. Th. Rüegg, L. Corley, C. B. Anfinsen, *J. Biol. Chem.* **251**, 1659 (1976); W. J. Jankowski, M. W. Davey, J. A. O'Malley, E. Sulkowski, W. A. Carter, *J. Virol.* **16**, 1124 (1975); M. W. Davey, E. Sulkowski, W. A. Carter, *J. Biol. Chem.* **251**, 7620 (1976); E. Sulkowski, M. W. Davey, W. A. Carter, *ibid.*, p. 5381.
9. M. Rubinstein, S. Rubinstein, P. C. Familletti, R. S. Miller, A. A. Waldman, S. Pestka, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
10. K. E. Mogensen and K. Cantell, *Pharmacol. Ther. A* **1**, 369 (1977); E. F. Wheelock, *J. Bacteriol.* **92**, 1415 (1966); K. Cantell and D. R. Tovell, *Appl. Microbiol.* **22**, 625 (1971).
11. S. Stein, C. H. Chang, P. Böhlen, K. Imai, S. Udenfriend, *Anal. Biochem.* **60**, 272 (1974).
12. P. Tucker and S. Pestka, *J. Biol. Chem.* **252**, 4474 (1977).
13. Obtained from Drs. G. J. Galasso and J. K. Dunnick of the National Institute of Allergy and Infectious Diseases, Bethesda, Md.
14. K. E. Mogensen and K. Cantell, *J. Gen. Virol.* **22**, 95 (1974).
15. We thank Dr. Sidney Udenfriend for enthusiastic support and guidance, Dr. Stanley Stein for making available instrumentation he developed for picomole assay of proteins and amino acids, Dr. K. Woods for helpful discussions, L. Brink for performing the exacting amino acid analyses, and D. Glover for assistance in interferon production.

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Design of Liposomes for Enhanced Local Release of Drugs by Hyperthermia

Abstract. *Liposomes can be designed to release an entrapped drug preferentially at temperatures attainable by mild local hyperthermia. In a test system in vitro, protein synthesis by* Escherichia coli *is inhibited and killing of the cells is enhanced by heating neomycin-containing liposomes to their phase transition temperature to maximize drug release. In the presence of serum the ratio of release at 44°C to that at 37°C can be made greater than 100:1, suggesting possible applications in the treatment of tumors or local infection.*

Liposomes are microscopic particles consisting of one lipid bilayer enclosing a single aqueous compartment (unilamellar vesicles) or a number of concentric bilayers enclosing an equal number of aqueous spaces (multilamellar vesicles). They are currently being studied as vehicles for delivery of pharmaceutical agents (*1*), but a major barrier to their use is the difficulty of directing them to specific target sites. The distribution of liposomes in vivo can be influenced somewhat by varying such nonspecific factors as the size, charge, fluidity, and route of administration, but not to a degree permitting flexible control of the site of delivery.

More specific "targeting" has been attempted by use of a "recognition macromolecule" to bind liposomes selectively to particular cells. Antibodies (*2–4*), plant lectins (*5*), and desialylated glycoproteins (*3*) have been tried, most often in model systems in vitro. This type of targeting faces special problems: the paucity of appropriate cell surface markers potentially useful for therapy; the possibility of immune sensitization of the host to the targeting moiety; the requirement in most cases that liposomes cross endothelial barriers; and the difficulty that bound liposomes do not always deliver their contents into the cells (*2, 4*). In this report we suggest a different approach, the use of local hyperthermia to promote selective delivery of liposome-encapsulated drugs to target areas.

Local hyperthermia is currently receiving increased attention as a therapeutic tool, for use either alone or in conjunction with radiation (*6*) or drugs (*7, 8*). Since many normal mammalian cells begin to show damage at about 42°C (*9*) the aim has been to achieve therapeutic results just a few degrees above physiological temperature. In addition to older methods of heating (for instance, in a warm bath or with warmed perfusate), microwaves and ultrasonic energy are now being investigated, especially for the local heating of deeper structures (*6*).

We can distinguish five ways in which local hyperthermia might increase the therapeutic effectiveness of drug-containing liposomes, for example in the treatment of neoplasms: (i) by promoting selective drug release at temperatures near that of the lipid phase transition of the liposomes; (ii) by increasing local blood flow; (iii) by increasing endothelial permeability to particles, thereby enhancing accumulation of liposomes in the target tissues; (iv) by increasing the permeability or susceptibility of target cells to the drug released from the liposomes; and (v) by increasing direct transfer of drug from vesicles to cells—for example, by fusion or endocytosis (*10*).

This study focuses primarily on the first of these possibilities. Near their liquid-crystalline transition temperatures (T_c), liposomes become highly leaky to water-soluble contents (*11*), a phenomenon generally attributed to disorder at the boundaries between solid and fluid domains in the lipid. Our basic strategy was to design liposomes with T_c above physiological temperature but in a range attainable by mild local hyperthermia. On passing through the heated area in the circulation, the liposomes would be expected to release their contents at a greater rate than elsewhere and thus to develop higher local concentrations.

Dipalmitoyl phosphatidylcholine (DPPC) appears to be a reasonable choice for the primary liposomal lipid. It has 16-carbon saturated fatty acid chains and a T_c of 41°C (*12*). By adding various proportions of distearoyl phosphatidylcholine (DSPC; 18-carbon chains; T_c = 54°C), it is then possible to obtain any desired T_c between 41° and 54°C. Since the two lipids are miscible in all proportions (in both "solid" and "fluid" states) only a single broad transition is observed (*13*). Small, primarily unilamellar vesicles of these lipids were prepared by a procedure involving sonication, Millipore filtration, and gel chromatography, essentially as described elsewhere (*14*) but with two important changes necessitated by the high T_c of the lipids: vortex mixing and sonication were done at 50° to 55°C,

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