presence of cells, but there were no mitoses in which chromosome complement could be identified. Some of these 11 embryos may have been the missing types of homozygotes. Inv(1) homozygosity apparently is not compatible with development. A lethal duplication or deletion, too small to detect by G banding, may have occurred during the creation of the interchange, or a position effect due to the chromosomal rearrangement could be the cause of lethality in the homozygous condition. The genetic imbalance created when either of the recombinant chromosomes is homozygous is also too severe for development.

This inversion in chromosome 1 has several interesting and unusual features: (i) The production of viable individuals with both types of recombinant chromosomes has apparently not yet been identified in other animals. (ii) Crossing-over can occur in a pericentric inversion in birds as contrasted to a situation where a pericentric inversion does not generate duplication and deficiency gametes, and reproductive efficiency seems to be minimally impaired (*9*). (iii) This inversion, with the different types of rearrangement carriers, is a promising model for the study of inversion recombination in vertebrates.

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Molecular Weight of Human Gamma Interferon Is Similar to That of Other Human Interferons

Abstract. *The molecular weight (as determined by molecular sieve chromatography) of human gamma interferon, formerly referred to as immune or type II interferon, is between 40,000 and 70,000. On sodium dodecyl sulfate–polyacrylamide gel electrophoresis, gamma interferon activity was recovered mainly from two regions of the gels corresponding to molecular weights of 20,000 and 25,000. The results suggest that in native form human gamma interferon may be aggregated.*

The three major interferon (IFN) species, designated alpha (leukocyte), beta (fibroblast), and gamma (immune) IFN's (*1*), are distinguishable on the basis of major antigenic differences (*2*). Amino acid sequences of human α-IFN and β-IFN have been determined partly by direct analysis of the purified proteins (*3*) and, more completely, by analysis of cloned complementary DNA sequences (*4*).

At least eight subspecies of α-IFN have been recognized by comparison of cloned DNA sequences (*5*); most α-IFN's do not appear to be extensively glycosylated (*6*). Beta interferon is glycosylated, but the exact size of the carbohydrate moiety has not yet been established. All α- and β-IFN genes analyzed so far code for 165 or 166 amino acid residues, with the molecular weights of the polypeptide moieties calculated to range from about 19,000 to 20,000 (*4*, *5*). Despite similarity in the number of amino acid residues, there is only about a 30 percent structural homology between α- and β-IFN polypeptides (*7*). In contrast, various subspecies of α-IFN show about 70 to 90 percent amino acid sequence homology (*5*).

Biological activity of α- and β-IFN's is not irreversibly destroyed by treatment with the anionic detergent sodium dodecyl sulfate (SDS). This property proved very useful for analytical work with various IFN's because separation of native IFN's by SDS–polyacrylamide gel electrophoresis (PAGE) can be monitored on the basis of biological activity eluted from sliced gels (*8*). Using this approach, many different laboratories reported β-IFN to be a homogeneously sized protein with an estimated molecular weight between 20,000 and 26,000 (*9*). Preparations of α-IFN generally exhibit a greater heterogeneity on SDS-PAGE, with biological activity associated with two or more size classes whose calculated molecular weights range between 15,000 and 23,000 (*6*, *10*, *11*).

Gamma interferon has many properties distinct from those of the other two major IFN species. It is produced by lymphocytes on stimulation with specific antigens or nonspecific mitogens (*12*) and differs somewhat from the other IFN's in its molecular mechanism of action (*13*). Generally, γ-IFN is held to be more important as an immunoregulator than as an antiviral agent (*14*). Unlike all other IFN's it is readily inactivated on exposure to *p*H 2 (*12*), and it has a higher isoelectric point (*15*, *16*). Earlier work with relatively crude preparations also indicated that, unlike α- and β-IFN's, γ-IFN is irreversibly inactivated by SDS treatment at 37° or 100°C (*17*), precluding molecular weight analysis by SDS-PAGE based on the recovery of biological activity from the gels after separation. Molecular weight determinations made on human γ-IFN by gel filtration indicate an apparent molecular weight that is significantly higher than that of other human IFN's determined by SDS-PAGE. The reported values are 50,000 (*18*), 40,000 to 46,000 and 65,000 to 70,000 (*19*), 58,000 (*16*), and 45,000 (*20*).

We observed that treatment of highly concentrated, partially purified γ-IFN preparations with 0.1 percent SDS at temperatures between 20° and 25°C did not completely destroy biological activity. This observation made possible SDS-PAGE analysis of the molecular weight of γ-IFN (Fig. 1). Biological activity was recovered from the gels at two peaks with apparent molecular weights of

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20,000 and 25,000. Traces of activity were also found in the region corresponding to a molecular weight of about 50,000.

With the aid of specific antiserums, the IFN activity recovered from SDS-PAGE was identified as being γ-IFN (Table 1). The fact that IFN activity recovered from the two major peaks was neutralized only by antibody specific for γ-IFN proves that this activity was not due to contamination with other IFN species.

Although the experience with α- and β-IFN's suggests that SDS-PAGE analysis reflects the true molecular weight of IFN's more accurately than gel filtration under nondenaturing conditions does, it is intriguing that molecular weight analysis of γ-IFN by gel filtration in many different laboratories yielded values that are approximately twice the molecular weights observed on SDS-PAGE. One possibility is that in its native form γ-IFN is an aggregate, possibly a dimer composed of two identical subunits. This interpretation is supported by the fact that some apparently undissociated IFN activity was found on SDS-PAGE in the 50,000 molecular weight range (Fig. 1) (other data not shown). Knight and Fahey (*21*) reported a 40,000 molecular weight form of human β-IFN that could be converted to the monomeric form by heating in SDS and thioglycolic acid. Since only approximately 10 percent of the original biological activity was recovered after SDS treatment (Fig. 1), we cannot rule out that the 20,000 and 25,000 molecular weight species represent minor γ-IFN components and that the bulk of γ-IFN activity, having a higher molecular weight, is irreversibly inactivated by SDS treatment. However, we consider this interpretation less likely than the notion that under denaturing conditions the high molecular weight form dissociates into the 20,000 and 25,000 molecular weight components. The fact that active protein could be recovered from two discrete regions of the gels should greatly facilitate the complete purification of human γ-IFN.

Our results suggest that γ-IFN may be closely related to the other IFN species in its molecular size. The observed molecular weight heterogeneity of γ-IFN on SDS-PAGE suggests that, like the other IFN species, γ-IFN's might comprise a multigene family of structurally related polypeptides. Alternatively, the heterogeneity might be due to some posttranslational modification such as variable carbohydrate contents. Virtually all human γ-IFN activity appears to be associated with glycoprotein (*16*).

Note added in proof: After the submission of this manuscript, Goeddel *et al.* (*24*) reported the cloning of human γ-IFN complementary DNA and its expression in three different host-vector systems. The nucleotide sequence of the cloned DNA indicates that human γ-IFN is composed of 146 amino acids with a total molecular weight of approximately 17,000. This value is compatible with our findings, since the molecular weight derived by Goeddel *et al.* does not include carbohydrate.

Table 1. Neutralization of IFN activity recovered after SDS-PAGE by antiserum to human γ-IFN. Two pools were prepared of material eluted from gel fractions 63 to 65 (25,000 molecular weight peak) and fractions 68 to 70 (20,000 molecular weight peak) from the experiment shown in Fig. 1. The pooled materials were incubated for 1 hour at 37°C with an excess of rabbit antiserum specific for α-, β-, or γ-IFN (*23*), or with a mixture of antiserums to α- and β-IFN. In parallel, the same antiserums were incubated with control IFN preparations diluted to contain several times the amount of IFN present in the analyzed fractions. Thereafter, residual IFN activity was determined as described in Fig. 1. N.D., not done.

Sample	IFN (unit/ml) in presence of antibody				
	None	To α-IFN	To β-IFN	To α- plus β-IFN	To γ-IFN
Peak at 25,000	128	96	128	128	< 4
Peak at 20,000	24	32	32	24	< 4
Control α-IFN	512	< 4	1024	< 4	1024
Control β-IFN	384	384	< 4	< 4	512
Control γ-IFN	512	N.D.	1024	768	< 4

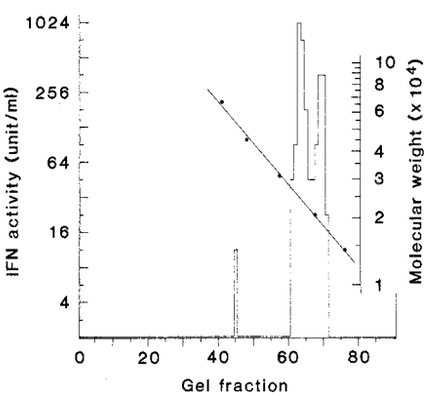

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of human γ-IFN. Human γ-IFN was produced in peripheral blood lymphocyte cultures in serum-free RPMI 1640 medium stimulated by combined treatment with 12-*O*-tetradecanoylphorbol-13-acetate and phytohemagglutinin (*17*). Purification of human γ-IFN was carried out by sequential chromatographic steps on controlled-pore glass and concanavalin A–Sepharose columns (*16*). The concanavalin A–Sepharose-purified sample was dialyzed exhaustively against 20 m*M* tris-HCl (*p*H 8.0); a suspension of DEAE-Sephacel equilibrated with the same buffer was then added for the absorption of contaminating proteins with isoelectric points at or below 8.0. The supernatant containing γ-IFN was collected after centrifugation at 1000*g* for 10 minutes and concentrated by dialysis against Aquacide II. The specific activity of this purified γ-IFN was estimated to be in excess of 1×10^7 units per milligram of protein. After dialysis against 8 m*M* tris-HCl (*p*H 6.8), the sample (6.9×10^5 unit/ml) was adjusted to contain 0.1 percent SDS (weight to volume) by adding a 10 percent SDS stock solution. After incubation at 25°C for 1 hour, the residual IFN activity remaining was 6.1×10^4 unit/ml (about 10 percent of the original titer). Electrophoresis was carried out with the Laemmli procedures (*22*) on a linear 10 to 16 percent gradient slab gel of acrylamide. Molecular weight standards (BioRad Laboratories) contained bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400). After electrophoresis the polyacrylamide gel was cut into 1-mm slices. Each slice was incubated for 24 hours at 25°C in 1 ml of Eagle's minimal essential medium supplemented with 5 percent (by volume) of heat-inactivated fetal bovine serum. Of the 6×10^3 units of IFN activity applied to the gel, 4.8×10^3 units were recovered from the gel slices. Interferon activity was determined by simultaneous assay in human GM-258 and FS-7 fibroblasts based on cytopathic effect of encephalomyocarditis virus (*17*). Similar activity profiles were obtained in GM-258 cells (as shown) and FS-7 fibroblasts (not shown). Interferon titers are expressed in laboratory units calibrated against an internal standard of γ-IFN.

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25. Parts of this work were presented at the International Meeting of the Biology of the Interferon System held in Rotterdam, 21 to 24 April 1981. Supported by PHS grants RO1-AI-07057 and RO1-AI-12948 and by grants from Flow General, Inc., Rentschler Arzneimittel GmbH & Co., Henry J. Kaiser Family Foundation, and Interferon Foundation. We thank M. Cassano, A. Feliciano, and L. Jashnani for technical assistance.

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Dynorphin Is a Specific Endogenous Ligand of the κ Opioid Receptor

Abstract. *In the guinea pig ileum myenteric plexus–longitudinal muscle preparation, dynorphin-(1–13) and the prototypical κ agonist ethylketocyclazocine had equally poor sensitivity to naloxone antagonism and showed selective cross protection in receptor inactivation experiments with the alkylating antagonist β-chlornaltrexamine. In binding assays with membranes from guinea pig brain, ethylketocyclazocine and dynorphin-(1–13) amide were more potent in displacing tritium-labeled ethylketocyclazocine than in displacing typical μ and δ opioid receptor ligands. In the two preparations studied, the dynorphin receptor appears to be the same as the κ opioid receptor.*

Subclasses of opioid receptors are distinguished on the basis of (i) differences in the physiological effects elicited by different opioids (*1*), (ii) differences in the relative potencies and sensitivities to naloxone antagonism of opioid agonists in different smooth muscle preparations (*2, 3*), and (iii) differences in the membrane binding characteristics of different opioids in vitro (*2, 4*). The opioid peptide dynorphin (*5–7*) appears to act through an opioid receptor different from that of the μ and δ types (*8, 9*).

The guinea pig ileum myenteric plexus is considered to contain μ and κ (but not δ) opioid receptors (*2*). The μ and κ receptors differ in their sensitivities to naloxone antagonism (*10*). We used the guinea pig ileum myenteric plexus–longitudinal muscle preparation to compare the four opioids [Leu]enkephalin (Leu, leucine), normorphine, dynorphin-(1–13) (D13), and ethylketocyclazocine (EKC) (a prototypical κ agonist) (Table 1). The equally high sensitivities of [Leu]enkephalin and normorphine to naloxone antagonism have been interpreted to mean that these two ligands act through the μ receptor in this tissue (*2*). Since D13 and EKC have equally low sensitivities to naloxone antagonism (*5*) (Table 1), the same reasoning suggests that D13 and EKC act through the κ receptor.

To investigate the relation between dynorphin and κ receptors, we used a method described in (*9*) to test whether D13 and EKC would show cross protection against the irreversible opiate receptor antagonist β-chlornaltrexamine (CNA) (*11*). Exposure of ileum longitudinal muscle strips (with attached myenteric plexus) to 3 n*M* CNA for 20 minutes resulted in parallel shifts of the log dose-response curves for the agonists, which were not reversed during the experiment (about 6 hours). The potencies of [Leu]enkephalin, normorphine, D13, and EKC were equally affected by CNA treatment (column 1 in Table 2).

For each reversible agonist, we determined the minimum concentration required, during CNA treatment, to reduce the potency shift by at least 80 percent. For selective protection, this minimum concentration was established in the tissue bath 1 minute before CNA was added. At the end of incubation, the tissue was washed to remove unreacted CNA and protecting ligand. The muscle strips were then tested with the agonists.

Receptor protection by D13 had no effect on the potency shift of [Leu]enkephalin or normorphine, but the potency shift of EKC was substantially reduced—to the same degree as that of D13 itself (column 2 in Table 2). Receptor protection by the stable enkephalin analog [D-Ala^2,D-Leu^5]enkephalin (Ala, alanine) (DADLE) reduced the potency shift of both [Leu]enkephalin and normorphine, but had no effect on the potency shift of D13 (column 3 in Table 2). The result with EKC was intermediate; its potency shift was significantly reduced ($P < .05$), but not to so great an extent as that of [Leu]enkephalin or normorphine. When EKC was used as the protecting ligand, equal protection was observed for normorphine, D13, and EKC itself (column 4 in Table 2). Receptor protection by normorphine showed a lack of selectivity similar to that of EKC (column 5 in Table 2). Protection provided by lower concentrations of EKC or normorphine showed less, but not selective, protection.

As shown by their naloxone sensitivities (Table 1), EKC and normorphine interact preferentially with the κ and μ receptors, respectively. However, at the saturating concentrations required for receptor protection against alkylation by CNA, each ligand also occupies the other receptor subclass, to which it has lower affinity. The results in Table 2 show that D13 is a more selective κ agonist than EKC and that DADLE is a more selective μ agonist than normorphine, although in mouse vas deferens this enkephalin analog is a selective δ agonist (*2*).

To characterize the receptor selectivity for dynorphin in guinea pig brain, we performed a series of competition binding experiments with [^{3}H]dihydromorphine ([^{3}H]DHM), [^{3}H]DADLE, and [^{3}H]EKC as primary ligands, and normorphine, DADLE, EKC, and D13 amide (*12, 13*) as competing ligands (for method, see legend to Table 3). Normorphine was much more effective against [^{3}H]DHM than against [^{3}H]DADLE and

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