a Sephadex G-150 column) and functional characteristics (for example, more marked effects on the dialyzable fraction of T_4 than on DFT_3 or RT_3U) of the thyroid hormone binding inhibitory activity in extrathyroidal tissues and that (*1, 2*) in the serum of patients with NTI. However, further study is needed to determine whether these factors are identical. The available data support the thesis that the inhibitory factor may leak from a tissue into the circulation in severe illness. The data also offer a possible explanation of the common clinical phenomenon of abnormal thyroid hormone levels in NTI (*1–3*). The use in our studies of a ratio of liver protein to total serum protein of about 1:7 to document a three- to fourfold increase in the T_4 dialyzable fraction of serum is of some concern, however. It may imply that there is actually an abundance of tissue proteins in the circulation of patients with NTI, or it may indicate that there are other factors that enhance the activity of the binding inhibitor in tissues in vivo.

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7. Incubation mixtures containing either radioactive T_4 and buffer or radioactive T_4, normal human serum (0.1 ml), and rat liver homogenate protein (4.5 mg) were dialyzed for 20 hours at 37°C. The dialysates were examined in two ways: (i) normal human serum was added to the dialysate and precipitation of radioactive T_4 by 10 percent trichloroacetic acid was studied; (ii) after an excess of a rabbit antibody to T_4 was added to the dialysate the mixture was incubated for 20 hours at 4°C and the antibody-bound radioactivity was separated from free radioactivity by means of goat antiserum to rabbit γ-globulin. In both studies, the proportion of radioactivity precipitated from the dialysates of incubation mixtures containing liver homogenate was 91 to 93 percent of that precipitated in the dialysates of buffer alone. These data suggested there was little breakdown of T_4 under the conditions of our assay for the T_4 dialyzable fraction.
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9. Radioactive T_4 diluted in 5 ml of 0.15*M* phosphate buffer (*p*H 7.4) was placed on one (A) side of a dialysis cell (Technilab, Inc.) in the absence and the presence of rat liver homogenate or HSA; plain buffer (5 ml) was placed on the other (B) side. After 20 hours of incubation at 37°C, the buffer from side B was removed and its content of T_4 was determined as described in (*4*). The dialyzable fraction in the presence of 4.5, 6.0, and 9.0 mg of liver homogenate protein was 95, 80, and 56 percent of that in the buffer, respectively, whereas that in the presence of 1.0, 2.0, and 4.0 mg of HSA was 59, 45, and 24 percent of that in the buffer, respectively. When plotted on a semilogarithmic plot, these data suggested that, on a weight basis, HSA was 6.5 times more potent in binding T_4 than liver homogenate protein. A value of 2.9 was obtained in a second experiment with a different liver homogenate.
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Recombinant Inversion Chromosomes in Phenotypically Normal Chickens

Abstract. *Some progeny resulting from interbreeding of individuals heterozygous for a pericentric inversion of chromosome 1 in the chicken have the two complementary types of recombinant chromosomes arising from a single crossing-over within the inverted segment. These individuals are capable of reproduction. Their progeny can have one or the other of the two recombinant chromosomes or, if crossing-over occurs, either a normal or an inversion chromosome.*

A fertile individual heterozygous for a pericentric inversion can produce gametes that have one of two morphologically distinct recombinant chromosomes as a result of crossing-over within the inverted segment. Individuals with such a recombinant chromosome occur in many species (*1, 2*). Progeny with one of these recombinant chromosomes are usually phenotypically abnormal because of the absence of portions of the chromosome containing the inversion. We do not know of any previous reports of viable vertebrates having both types of complementary recombinant chromosomes.

When individuals heterozygous for a pericentric inversion in chromosome 1 [inv(1)] (*3*) of the chicken (*Gallus domesticus*) were mated, some progeny—designated rec(1)—had both recombinant chromosomes. These two types of chromosomes are apparently completely complementary, since the carriers are phenotypically normal and fertile when sexually mature. Furthermore, the interbreeding of rec(1) carriers may give rise to normal, inversion, or either of the recombinant types of chromosomes, because of crossing-over during meiosis.

The original inversion was induced by injection of ethyl methanesulfonate into a mature male (*4*). A daughter of this male identified as having the inversion was outcrossed, for development of the rearrangement line, to a male with standard chromosomes. Progeny were examined for their chromosome complement by use of the feather pulp technique (*5*). Some of the progeny from subsequent interbreeding had the two complementary types of duplication and deficiency recombinant chromosomes that had arisen from a single crossing-over within the inverted segment. Trypsin-Giemsa banding (G banding) (*6*) (Fig. 1) indicated that the longer chromosome was duplicated for approximately two-thirds of the p arm, and this was attached to the distal end of the q arm. The shorter chromosome did not have this same segment. A small portion of the terminal region of the q arm, designated F in Figs. 2 and 3, appears to be duplicated on the p arm of the shorter (1p−q+) chromosome and missing on the longer (1p+q−) chromosome.

Two different meiotic configurations occurred in inv(1) males, in about equal frequencies (Fig. 1). Interpretations of these configurations are given in Fig. 3, a and b. The open-loop diakinesis configuration (Fig. 1, lower left) suggests that the entire inverted segment is unpaired, preventing any crossing-over in this region. Only inv(1) and normal chromosomes can be recovered from this configuration. The figure-eight diakinesis configuration (Fig. 1, lower right) shows an apparent reversed chiasma such as that described in the lily (*7*), where homolo-

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gous pairing is achieved in the central portion and crossing-over can occur. A single crossover in this region would result in potential recovery of all four types of chromosomes: normal, inversion, 1p−q+, and 1p+q−. This type of meiotic configuration has also been described in humans (*2*).

Two types of meiotic configuration were also seen in rec(1) males, in unequal frequencies. Most of the configurations (> 90 percent) were of the figure-eight type shown in Fig. 1; the rest were of the open-loop type. To achieve the figure-eight configuration, the two recombinant chromosomes must undergo partial pairing, as diagramed in Fig. 3c. Synapsis occurs in only a portion of the inverted segment, allowing crossing-over and recovery of all four types of chromosomes, with asynapsis in the rest of the configuration. Incomplete synapsis similar to this has also been described in *Drosophila melanogaster* (*8*).

Cytological screening has not identified any individuals homozygous for the inversion chromosome or for either recombinant chromosome among more than 500 chicks hatched from various combinations of inv(1) and rec(1) matings. When rec(1) heterozygotes are mated to either rec(1) or inv(1) heterozygotes, the progeny are normal, inv(1), or rec(1). When rec(1) heterozygotes are mated to normal individuals, only inv(1) heterozygotes and normal progeny are recovered. Early embryos homozygous for the inversion (as recognized by the misplaced centromere) are slow in development and die within 24 to 48 hours of incubation.

A cytological study (*6*) of early embryos also indicated that if normal or inv(1) chromosomes are paired with either of the unbalanced recombinant chromosomes, death of the embryo occurs within 24 to 48 hours of incubation. Positive identifications were made on 33 embryos from inv(1) × inv(1) and rec(1) × inv(1) matings. Two of these embryos were inv(1) homozygotes, eight were inv(1)/1q+q− heterozygotes, and four each were 1p−q+/1p+q−, 1p−q+/N (N, normal), and 1p+q−/N heterozygotes. These were slow in development at 24 hours of incubation. The other 11 embryos were normal or inv(1)/N heterozygotes. No 1p+q− or 1p−q+ homozygotes were found. At 24 hours of incubation 11 embryos were dead; slides prepared from these embryos verified there was some development, as shown by the

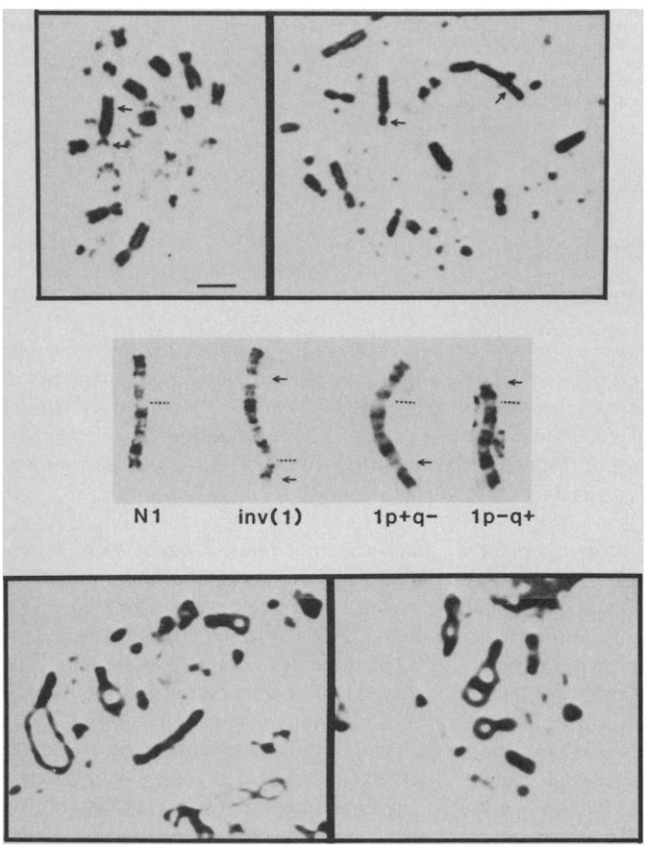

Fig. 1. Mitotic spread from heterozygous inv(1) (top left) and rec(1) (top right) carriers. G banding of the three types of rearranged chromosomes are compared to the normal chromosome 1 (center row). Dotted lines indicate centromere position and arrows indicate break-reunion points. Diagrammatic interpretation of break-reunion points is shown in Fig. 2. Two types of meiotic configurations can be recovered from both inv(1) and rec(1) males (bottom). Diagrammatic interpretation of these configurations is shown in Fig. 3. Scale bar (top left), 5 μm.

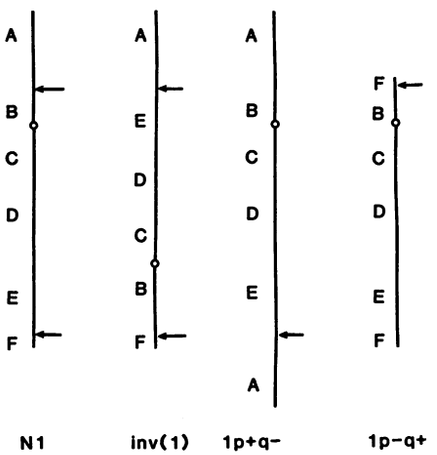

Fig. 2 (left). Diagrammatic representation of the four types of chromosomes discussed in the text. Arrows indicate the break-reunion points.

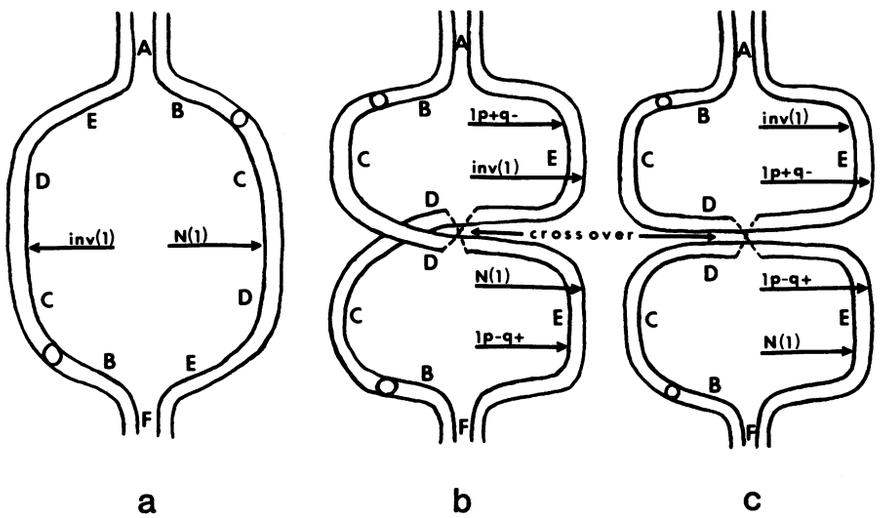

Fig. 3 (right). Diagrammatic representation of meiotic configurations in Fig. 1. (a) Interpretation of diakinesis in lower left of Fig. 1, where the inverted segment is unpaired. (b) Interpretation of diakinesis shown in lower right of Fig. 1, where a half twist of (a) allows a portion of the inverted segment to pair homologously. This will allow crossing-over and generation of recombinant chromosomes. (c) Interpretation of figure-eight diakinesis configurations from rec(1) males, where crossing-over will generate the four types of chromosomes discussed in the text.

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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