were incorporated into glandular proteins in cortisol-treated than in control glands. Cortisol-treated glands also had higher immunoreactive PTH content than control glands, though the differences were not statistically significant. Phenol extracts of glands chromatographed on Biogel P-10 in 3*M* guanidine hydrochloride and 2.3*M* formic acid yielded a substance with a consistent small peak of radioactivity for which the partition coefficient, K_d, was 0.18 to 0.2, the same as that for highly purified bovine PTH labeled with ^{125}I. If one assumes that this ^{3}H-labeled peptide is ^{3}H-labeled rat PTH (P-10–rPTH), the cortisol-treated glands also contained 30 percent more ^{3}H-labeled P-10–rPTH per microgram of dry tissue than the control glands.

These results suggest that cortisol stimulates the synthesis of proteins and PTH. Although the changes in the glandular content of ^{3}H-labeled proteins and PTH were relatively small, they were sufficient to prevent decreases in glandular hormone content during 3 days of culture when PTH secretion was stimulated. This reflects a rapid turnover rate for PTH synthesis and secretion which has been previously observed in ultrastructural studies of rat parathyroid glands (*9*) as well as in short-term studies in vitro with bovine parathyroid gland slices (*10*). It is possible that cortisol acts indirectly to increase net PTH synthesis either by increasing active membrane transport of amino acids or by decreasing the rate of intracellular degradation of PTH. Habener and co-workers have proposed that the control mechanism for the secretion of PTH in bovine parathyroid gland slices is mediated through a calcium control of intracellular degradation of newly synthesized PTH; a high concentration of calcium in the medium decreases the secretion rate by increasing the rate of PTH degradation (*11*). Cortisol can thus affect secretion through inhibition of calcium uptake by the parathyroid gland, analogous to its ability to inhibit the absorption of calcium in the gut (*12*). It is also possible that cortisol, through its known ability to stabilize lysosomal membranes and decrease protease release (*13*), could decrease intracellular PTH degradation and thus increase secretion.

The specific relative effect of glucocorticoid congeners on parathyroid hormone secretion, and the findings that cortexolone alone has no effect but can inhibit the effects of cortisol, are consistent with the presence of cortisol receptors in the parathyroid cell (*14*). The extremely small size of each rat gland (15 to 30 μg dry weight) makes the isolation and demonstration of specific receptors in these glands technically difficult.

This study provides another explanation for the increase in plasma PTH in cortisol-treated animals and suggests that some of the effects of therapeutic doses of cortisol to decrease bone mass might be mediated through this action. Thus, in cortisol-induced osteopenia the direct stimulation of PTH secretion by cortisol might overcome the inhibitory effect of cortisol on bone resorption (*15*). An additional effect from secondary stimulation of parathyroid hormone secretion could also result from hypocalcemia produced by cortisol inhibition of gut calcium absorption (*12*) and bone resorption (*15*). The net result would be an increase in the concentration of PTH in the plasma, normocalcemia, and increased bone resorption (*16*) in the parathyroid-intact animal treated with cortisol.

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Complete Covalent Structure of a Human IgA1 Immunoglobulin

Abstract. *The complete covalent structure has been determined for a human myeloma IgA1 immunoglobulin. This protein has unique features in the amino acid sequence and disulfide bridge structure of the variable (V) and constant (C) regions of both the α heavy and the λ light chains, and in the number and loci of oligosaccharides. Whereas C region domains of heavy chains have evolved independently over eons, recent isotypic variations have occurred in λ light chains and possibly in α heavy chains.*

Despite the vast amount of partial sequence data for human immunoglobulins (*1, 2*), until recently the complete covalent structure had been reported for only one IgG molecule (*3*) and two IgM molecules (*4*). We here report the complete covalent structure of a human IgA1 myeloma globulin (designated Bur) including the complete amino acid sequence of the α1 heavy chain, the λ light chain, the location and kind of the oligosaccharides, and the probable disulfide bridge structure (Fig. 1). In the constant (C) region of the α1 chain our sequence differs in only 8 positions out of 353 from another α1 chain (Tro) recently reported (*5*) in which the location of the carbohydrates and disulfide bridges was not given. However, there are 42 differences in the variable (V) regions of the two α1 chains, excluding differences in amide assignments, gaps, and the presence of carbohydrate in the V region of the Bur α1 chain. These studies verify the schematic structure we have previously proposed for human IgA1 immunoglobulin (*2, 6*); they validate, extend, and correct many partial sequences of portions of the human α1 chain proposed by other workers (*7*) and the complete Fcα sequence reported by us (*8*). The λ light chain of IgA1 Bur has three unusual positions of variation in the C region, independently described by us (*9*) and by Fett and Deutsch (*10*). In both the light and the heavy chains of IgA1 Bur, the switch point (the division between the V and C regions of chains of the same class) extends into what was previously regarded as the C region, thereby casting doubt on the existence of a nucleotide sequence that signals the point of union for the postulated V and C genes (*2–9*).

The order of the 472 amino acid residues of the Bur α1 chain was determined by automatic or manual sequence analysis of more than 200 different peptides from the whole α1 chain, its CNBr frag-

ments, and the Fc fragment produced by IgA1 protease (*8*). These included: (i) tryptic, chymotryptic, and thermolytic peptides of the reduced and carboxymethylated α chain, (ii) tryptic peptides of the carboxymethylated Fd and Fc fragments, (iii) tryptic peptides of the aminoethylated succinylated α chain, and (iv) tryptic peptides of the aminoethylated Fd fragment. Integration of the results yielded a unique sequence with placement of all amino acids and overlapping of all peptides. The oligosaccharides were placed by sequence analysis of glycopeptides. Disulfide bridges were deduced from the results of reduction-alkylation, aminoethylation, enzymatic cleavage, and CNBr fragmentation and by homology to results of other workers (*7, 11*).

IgA is unusually rich in half-cystine compared to other classes of immunoglobulins that have been sequenced; the α1 chain has 17 half-cystine residues compared to 11 in γ1, which has four domains, and compared to 14 half-cystine residues in μ and 15 half-cystine residues in ϵ, both of which have a fifth domain. The function of some of the half-cystines in IgA is not clear. However, the two ex-

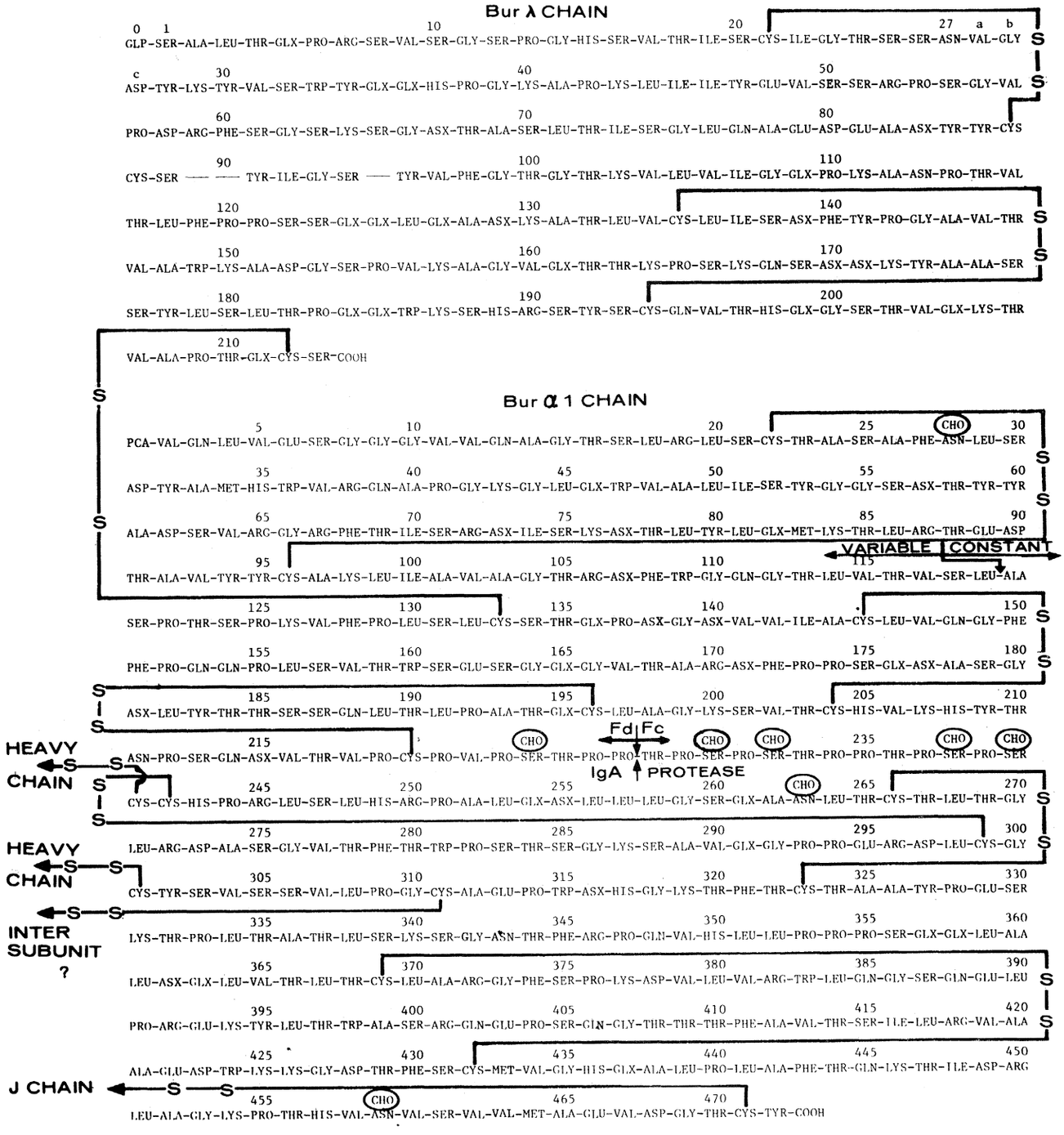

Fig. 1. Complete covalent structure of the half-molecule of the monomer form of the human IgA1 myeloma globulin Bur. The amino acid sequence of the λ light chain is given at the top and that of the α1 heavy chain below. The intrachain disulfide bridges characteristic of the domains are at the right and the interchain bridges and other intrachain bridges at the left. CHO denotes carbohydrate. The division into the variable and constant regions of the α1 chain and the point of cleavage into Fd and Fc by IgA1 protease are shown by arrows. The light chain is numbered according to the numbering system for Sh, with use of 27a, b, and c for insertions in the first hypervariable region and gaps at positions 89, 90, and 95 to maximize the homology to other λ chains.

tra intrachain disulfide bridges in the hinge region (Cys-196 to Cys-220, and Cys-242 to Cys-299 in Fig. 1) probably contribute to resistance of IgA to proteolytic cleavage in extracellular fluids such as intestinal secretions, where IgA is the predominant immunoglobulin class.

We have shown (*8*) that the α, γ, μ, and ϵ chains have strong homology in amino acid sequence (about 30 percent) distributed nonuniformly throughout the Fc region; μ and α are least alike (20 percent) in the first domain of Fc and are most alike (50 percent) in the second. In the first domain of Fd, Cα1 is equally homologous to Cγ1, Cμ1, and Cϵ1 (33 percent), but of all combinations Cγ1 and Cϵ1 are most alike (45 percent). However, although most alike as a set, any of the first C_H1 domains is almost equidistant from any of the C_H2, C_H3, or C_H4 domains of any other of the four heavy chains; that is, the average homology is about 25 percent. Thus, over eons the individual domains of heavy chains have evolved independently rather than through a line of descent in which one whole chain is the ancestor of another.

There has been no previous evidence for sequence differences in the α1 C region that are isotypic (two or more subclasses present in all normal individuals) or allotypic (two alleles at one locus). It is uncertain whether any of the eight differences in the C region sequence of the Bur and Tro α1 chains are due to isotypic or allotypic differences or to sequence errors, but at least three differences appear to represent real interchanges (*12*). These are Glu-295, Pro-309, and Thr-409 in Bur α1 versus Asx-295, Ser-309, and Glu-409 in Tro α1. Pro-309 is also reported in α1 Oso and α2 Avi (*11*), and we have found Thr-409 in α1 Ha (*13*). The five other differences are technical in nature and probably do not reflect genetic changes (*12*).

The most significant features of the Bur λ light chain are as follows. (i) It is of the $V_{\lambda II}$ subgroup; (ii) it has the "Mcg substitutions" in the C region; and (iii) all 13 replacements in the V region relative to Mcg are compatible with the three-dimensional structure of the Mcg light chain (*14*). The Mcg substitutions in the C region are Asn-113 for Ala-113, Thr-115 for Ser-115, and Lys-164 for Thr-164 (*15, 16*). The Bur and Mcg λ chains are identical in their C regions being Mcg/Bur^{+3}, Oz$^-$, Kern$^+$ in their isotypic markers; they thus differ by as many as five residues from the other Cλ isotypes. This supports the suggestion that the Mcg sequence represents a new Cλ gene (*10*).

In the V region the Bur λ chain has several unusual features. There is a Cys-Cys sequence at positions 86 and 87; Cys-86 is assumed to form the typical intrachain disulfide bond with Cys-21. The function of Cys-87 is unknown. Cys-87 is assumed to be a free but unreactive sulfhydryl group, since it is not converted to carboxymethylcysteine during the mild reduction and alkylation used to separate H and L chains. Neither is it reactive to *N*-dansylaziridine, either in the presence or absence of 6*M* urea.

Comparison to the three-dimensional structure of Mcg shows that the changes in the third hypervariable region of Bur are located in positions that are either oriented toward the exterior of the V_L domain or, like Cys-87, project into the binding cavity. A similar Cys-Cys sequence is present at positions 86 and 87 in the λ light chain of Tro IgA1 (*5*) where the function is also unknown.

All the carbohydrate of the C region of the α1 chain is in the hinge region and the Fc fragment. Baenziger and Kornfeld (*17*) have determined the structure of the two kinds of oligosaccharide units that are present. (i) Several complex branched oligosaccharide units linked to asparagine via *N*-acetylglucosamine; (ii) five small oligosaccharide units containing *N*-acetylgalactosamine linked via the *O*-glycoside to serine in the hinge region. We have determined that there are only two glucosamine oligosaccharides in the Cα1 region, one linked to Asn-263 and one to Asn-459. In addition, because of the adventitious presence of the tripeptide acceptor sequence Asn-Leu-Ser in the first hypervariable region of the Bur V_H, a third glucosamine oligosaccharide is attached to Asn-28. The tripeptide acceptor sequence is Asn-X-Ser/Thr, where X is any residue, and the third residue is either serine or threonine (*17*). Though the carbohydrate in the V_H region is uncharacteristic, its presence in Bur α1 is not unique and is due to the recognition by transglycosidases of the acceptor sequence. This helps to explain reports of variable carbohydrate content in human IgA1 proteins.

There is no unique sequence common to the COOH-terminus of the V_H region of human α, γ, and μ heavy chains that acts as a signal point or switch sequence for V-C joining. The C region of α1 chains begins at Ala-120 with Ala-Ser-Pro; this sequence is present in α1 Tro and several other α1 chains for which only partial sequence data are available (*7*). The C regions of the γ and μ chains begin at a comparable position with Ala-Ser-Thr and Gly-Ser-Ala, respectively. Although Val-Ser-Ser at first had appeared to be a switch point common to the COOH terminus of the V_H region of all heavy chains (and is present in α1 Tro), one substitution has been noted in the valine (μ chain Ga) (*18*) and one in the second serine (μ chain Gal) (*4*). In α1 Bur the sequence is Val-Ser-Leu (positions 117 to 119). Thus, the switch point of α, μ, and γ heavy chains does not contain a common nucleotide sequence that acts as a signal for union of the V_H and C_H genes.

The Vλ-Cλ switch region in human λ light chains begins at Gln-109 with the sequence Gln-Pro-Lys-Ala (*16*). The presence of two substitutions in the sequence immediately following (Asn-Pro-Thr in Bur and Mcg versus Ala-Pro-Ser in other λ chains) might appear to extend the variable region. However, since the Mcg/Bur substitutions are coupled with another substitution at position 164 (lysine for threonine) and with the Oz$^-$ and Kern$^+$ isotypic markers, we agree with Fett and Deutsch (*10*) that the Mcg/Bur sequence denotes a separate Cλ gene.

In sequences of the length of the C region of the α1 chain, it is remarkable that such few differences appear from independent laboratories. Although some of these differences may be attributable to technical problems, several appear real. These results indicate the importance of complete sequence analysis of immunoglobulins and emphasize the possibility of greater variation in the C region of both light and heavy chains than had previously been suspected. Because this has import for theories of the recent evolutionary history of C region genes, further search for isotypic or allotypic variation in α1 chains is warranted.

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1. For the nomenclature of immunoglobulins and schematic diagrams of their polypeptide chain structure and of the Fab and Fc pieces of immunoglobulins A, M, and G (IgA, IgM, and IgG), see F. W. Putnam (*2*). Abbreviations of the amino acid residues are as follows: Lys, lysine; His, histidine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Asx, aspartic acid or asparagine, identity not established; Thr, threonine; Ser, serine, Glu, glutamic acid; Gln, glutamine; Glx, glutamic acid or glutamine, identity not established; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan.
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15. Because of variability in length of the V region, identical residues in the C region of immunoglobulin polypeptide chains of the same class may differ in number if a consecutive numbering system beginning with the NH_2-terminus is used. In order to retain the same position number for identical residues a numbering system was proposed, based on Sh, the first human λ light chain to be completely sequenced (*16*). A different convention was used for the Mcg λ chain, which has 216 residues compared to 214 in Bur λ and 213 in Sh λ. Thus, the C region substitutions numbered in Mcg as Asn-116, Thr-118, and Lys-167 correspond to Asn-113, Thr-115, and Lys-164 in Bur λ. Although the Bur and Tro α1 chains differ in the number of residues in the V region (119 and 122, respectively), they have the same numbering system in the C region.
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Histone Transition During Spermiogenesis Is Absent in Segregation Distorter Males of *Drosophila melanogaster*

Abstract. *Males homozygous for the segregation distorter chromosome are often sterile or nearly sterile as a result of the dysfunction of virtually all their sperm. Spermatid bundles from such males do not exhibit the normal transition from lysine-rich to arginine-rich histones.*

Natural populations of *Drosophila melanogaster* typically contain a low frequency of certain second chromosomes designated SD (segregation distorter) (*1*). Heterozygous SD/SD$^+$ males produce mostly SD-bearing offspring because the sperm carrying SD$^+$ undergo dysfunction (*2*). Homozygous SD/SD males are sterile or nearly sterile as a result of the dysfunction of virtually all their sperm (*3*).

The developmental lesion leading to sperm dysfunction is not known. Motivated by suspicion that the lesion might involve the transition from histones rich in lysine to histones rich in arginine during spermiogenesis (*4*), we examined the histone transition in sterile SD/SD males. Here we report direct cytochemical evidence that the transition from somatic histones to sperm histones does not take place.

Two lines of circumstantial evidence focus attention on the histone transition. The first is a genetic correspondence. Segregation distorter chromosomes are known to carry two mutations, one called *Sd* (for segregation distorter), the other called *Rsp* (responder) (*5*). The *Sd* locus is on the left arm of the second chromosome near the locus of purple (2 : 54.5) (*5*). The histone locus is in region 39D-E of the salivary gland chromosomes (*6*), also close to purple. While an identity between the histone locus and *Sd* has not been shown, these observations suggest that the loci might be genetically or functionally related. A second line of circumstantial evidence implicating the histone transition is cytological. Heads of dysfunctional sperm often fail to condense; when they do condense, they appear to be unstable and frequently undergo a characteristic sequence of stages of degeneration (*7*). This, too, is consistent with the hypothesis that the developmental lesion involves a defective transition from lysine-rich to arginine-rich histones during spermiogenesis, since the histones rich in arginine are generally assumed to function in sperm head condensation (*8*).

Our results are shown in Table 1. Because SD-bearing chromosomes from nature carry recessive lethals unrelated to segregation distortion, SD homozygotes must be produced by combining SD's from different populations. The SD chromosomes listed in Table 1 were all isolated from natural populations in the midwestern United States (*9*), except for SD(NH)-2, which was isolated in Japan (*10*). Late spermatid bundles (*11*) were stained either for all histones (those rich in either lysine or arginine) or specifically for histones rich in arginine (*12*).

The first genotype examined was SD-72/SD(NH)-2. Histones rich in arginine were found to be absent in spermatid bundles, but total histones appeared normal. Since the males were 3 to 5 days old at the time of examination, the result could have been due to a delayed histone transition and not to its absence. Males

Table 1. Absence of arginine-rich histones in late spermatid bundles of homozygous SD males and correlations with male fertility. Fertility of males is taken from Hartl (*9*) except where noted in text. Cytochemical histone assay was modified from Alfert and Geschwind (*12*) and Das *et al.* (*4*). Testes of males dissected in Ringer were air dried, fixed with 10 percent formaldehyde for 6 hours, washed in tap water overnight, treated with 10 percent trichloroacetic acid for 30 minutes at 90°C, washed in 70 percent ethanol, washed in distilled water, and stained for 2 hours with 0.1 percent fast green at *p*H 8.1; the testes were then dehydrated in an ethanol series and mounted in Permount. Specific staining for histones rich in arginine was the same except for deamination of lysine residues with 5 percent $NaNO_2$ for 30 minutes after the treatment with trichloroacetic acid. In all cases, testes from wild-type males were placed on the same slides as those from experimental males to serve as controls. Testes from wild-type males were stained both for total histones and for histones rich in arginine. Each result is based on examination of testes from more than 60 males in at least two separate experiments.

Genotype of male	Fertility of male	Histones in late spermatid bundles	
		Lysine-rich plus arginine-rich	Arginine-rich
SD-72/SD(NH)-2	−	+	−
SD(M)-35/SD-72	−	+	−
SD(43)-1/SD-72	−	+	−
SD(M)-35/SD(NH)-2	−	+	−
SD(Berea)-110/SD(NH)-2	−	+	−
SD(43)-1/SD(NH)-2	−	−	−
SD(Berea)-110/SD-72	+	+	+