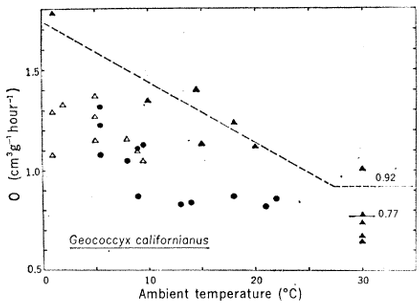

Fig. 3. Relation between oxygen consumption and ambient temperature in adult roadrunners. The dashed line is from data of Calder and Schmidt-Nielsen (*8*). Symbols: ▲, in dark; △, in dark, hypothermic; ●, posturing in "sun."

ported for a number of desert-dwelling birds (*12*), although the roadrunner is one of the largest species in which this phenomenon has been adequately demonstrated.

Our results confirm the hypothesis of Hamilton and Heppner (*1*) that birds may utilize sunlight to reduce their energy expenditures because substantial energy savings were observed in sunning roadrunners. Under the conditions of our study, the average energy saving was 551 cal hr^{-1} (*13*), an amount equivalent to 41 percent of the standard metabolism predicted for a 294.6-g bird (*14*).

Heppner (*15*) suggested that in zebra finches (*Poephila castanotis*) dyed black and exposed to artificial radiation "warming of the outer feathers . . . reduces the temperature gradient from the skin of the bird to the surface feather, and thus slows the loss of metabolic heat to cold surroundings." This mechanism may operate in the exposed soft black plumage of sunning roadrunners. However, sunning roadrunners also expose the naked black skin of the interscapular region, and this area appears to absorb solar radiation directly. In birds that are not sunning, the naked skin is insulated by the plumage of the cervical and scapular tracts.

Previous studies of thermoregulation, evaporative water loss, and metabolism of roadrunners have been interpreted as indicating that the species is no better adapted to desert conditions in these functions than pigeons and doves (*8, 16*). Recent data indicate that roadrunners possess a number of physiological specializations which are advantageous for a desert existence; these include a salt-secreting nasal gland (*17*), a capacity for water conservation when water is limited (*18*), and adaptations for energy conservation through hypothermia and solar absorption.

The fact that selective forces have produced such specialized behavior and morphology for absorption of sunlight by roadrunners indicates that this phenomenon has ecological significance. Sunning behavior could effectively substitute calories from solar input for those derived from biochemical processes. The inability of roadrunners to make sustained flights to gather food combined with the reduction of insect numbers in the winter months must exert considerable selective pressure for energy conservation. Bryant (*19*) reported that vegetable materials in the diet of roadrunners increased from 3 to 10 percent during the winter months. Thus, even though sunning occurs throughout the year, it is probably most important during the winter when the productivity of the desert is low.

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5. An Eppley pyranometer (model 8-48) was placed in the same position and approximately at the same height as the back of the posturing roadrunner. The output of the pyranometer was monitored with a Sargent SR recorder.
6. Thirty-gauge copper-constantan thermocouples were used in conjunction with a Honeywell Electronik 16 recorder.
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Structure of the Hinge Region of the Mu Heavy Chain of Human IgM Immunoglobulins

Abstract. *The amino acid sequence around the central disulfide bridge linking the mu heavy chains of the human immunoglobulin M monomer is unlike that in immunoglobulin G. This hinge area contains one of the five oligosaccharides of the mu chain, is low in proline, and is the site of tryptic cleavage to yield Fabμ and Fcμ fragments.*

Immunoglobulins of the three major classes (IgG, IgA, and IgM) have a common tetrachain structure composed of a pair of heavy chains (γ, α, and μ, respectively) and a pair of light chains (κ or λ), but IgG, IgA, and IgM differ from each other in many properties because each heavy chain has a characteristic sequence in its constant region (*1*). For example, owing to an intersubunit disulfide bridge on the μ chain, IgM exists predominantly as a 19*S* pentamer of the tetrachain subunit, while IgG, which lacks such a bridge, is a 7*S* monomer. Whereas human IgG is very susceptible to limited cleavage by papain at 37°C to yield two well-defined pieces (Fab and Fc) (*2*), human IgM is rapidly degraded to heterogeneous fragments and peptides (*3*). This difference is attributable to the nature of the primary and tertiary structure of the two proteins especially in that portion of the heavy chain (the hinge region) that couples the Fd and Fc segments of each of the two classes of heavy chains (μ in IgM and γ in IgG). The hinge region has been operationally defined as a section near the middle of the heavy

chain which is uniquely susceptible to proteolysis, relative to other parts of the molecule. Electron microscopic observations have been interpreted to show that both IgG and IgM are flexible in this region. In human and rabbit IgG, this flexibility or open nature has been attributed to the presence of the large number of proline residues in the approximate midpoint of the γ chain (*4, 5*) (see Fig. 1).

We report here the amino acid sequence of 44 residues in the hinge region of the human μ chain (Fig. 1) and show that it lacks the proline-rich sequence and two of the interchain disulfide bridges that are characteristic of the human γ1 chain (*6, 7*) and that are believed to render this segment of the γ chain singularly exposed to enzymatic attack. Unlike the γ1 chain, the μ chain has a large carbohydrate group in the hinge region. As in some rabbit γ chains (*8*), this oligosaccharide may interfere sterically with the action of proteolytic enzymes. However, by incubation with trypsin at 56° to 60°C, human IgM is converted in high yield to a Fabμ fragment of 3.6*S* and a decameric Fcμ fragment of 10*S* (*9*). We have found that this cleavage occurs specifically at an arginine peptide bond that is six residues away from the oligosaccharide, and we attribute this acquired sensitivity to a temperature-dependent conformational change exposing the susceptible bond. The preparation of these fragments has greatly facilitated our sequence determination of the entire μ chain, as well as of the hinge region.

The sequence of the hinge region of the μ chain of the IgM globulin Ou (Fig. 1) was assembled from data obtained by conventional amino acid sequence analysis of some 20 purified peptides and by analysis with the automatic amino acid sequenator of the Fcμ fragment and of F2(Fcμ), a large fragment of the μ chain obtained by cleavage with CNBr (*10*). The peptides were prepared by digestion of the whole μ chain or of its CNBr fragments with thermolysin or with trypsin (*10, 11*) or by digestion of the Fcμ fragment with chymotrypsin. Altogether, more than 200 peptides have been completely or partially sequenced in our study of the structure of the μ chain. In addition to the methods of sequence analysis already described (*12*), the dansylation procedure (*13*) was used for peptides. The large Fcμ and CNBr fragments were subjected to the automatic Edman degradation method (*14*) with the Beckman Sequencer model 890. The phenylthiohydantoin derivatives of the amino acids were identified by thin-layer chromatography and by gas chromatography with a combination of two different columns (*15*). The Fabμ and Fcμ fragments were prepared by digestion with trypsin at 60°C and were separated on Sephadex G200. Their homogeneity was checked by immunoelectrophoresis, immunodiffusion, and ultracentrifugation. The carbohydrate content of the glycopeptide was determined by gas chromatography (*16*). The oligosaccharide had a complex structure and contained fucose, mannose, galactose, *N*-acetylglucosamine, and sialic acid.

Figure 1 shows the sequence of a 44-residue segment of the human μ chain, including the site of tryptic cleavage at 60°C in comparison with the sequence of the hinge regions of the human γ1 chain and the rabbit γ chain. Only the human γ1 chain is numbered because the sequence of both the human μ chain and the rabbit γ chain is incomplete in the highly variable region around position 105 just after the first intrachain disulfide bridge. Various human γ1 chains differ in length in the variable region (*6, 7*). However, it is convenient to refer to a residue in the human μ and the rabbit γ chains by giving the number of the homologous residue in the constant region of the human γ1 chain (*7*) to which it is compared. Our positioning of the hinge region of the μ chain is based not on homology, which is slight, but rather on our total sequence information on the Fabμ fragment. This indicates that the hinge region sequence for the μ chain shown in Fig. 1 begins at or close to residues 215 to 220 and thus closely corresponds in location to the hinge regions of the human γ1 and rabbit γ chains. In order to avoid confusion we have deferred numbering the μ chain until the sequence is complete.

The μ chain sequence includes a 21-residue tryptic glycopeptide designated μ2 by Beale and co-workers (*17*). Our sequence for μ2 agrees exactly with their amino acid composition. Both Beale *et al.* and Miller and Metzger (*18*) showed that μ2 contained one of the four interchain disulfide bridges and gave evidence that it forms the bridge between the heavy chains which links two Fab'μ fragments to form the (Fab')$_2\mu$ fragment derived by prolonged tryptic digestion at 37°C. By stepwise labeling with ^{14}C-labeled alkylating agents (*11*) we were able to show that the NH_2-terminal cysteine of CNBr fragment F2(Fcμ) forms an interchain disulfide bridge rather than an intrachain bridge. Because this cysteine is at the beginning of the F2(Fcμ) fragment, the interchain bridge which it forms must be between two heavy chains rather than between a heavy and a light chain. This cysteine was recovered as [^{14}C]carboxymethylcysteine after limited reduction in mercaptoethanol and alkylation with [^{14}C]iodoacetic acid. In contrast, other cysteine residues involved in intrachain bridges were recovered as [^{14}C]aminoethylcysteine after further reduction in 7*M* guanidine hydrochloride and alkylation with [^{14}C]ethylenimine. However, in our work with trypsin at 60°C, μ2 is the NH_2-terminal peptide of the polymeric Fcμ fragment. This was shown by direct sequence analysis of the Fcμ fragment with the automatic sequencer for 20 steps. Although the yield was low at several steps involving threonine or the dicarboxylic acids, overlapping was established by 22 steps of Edman degradation on the large fragment F2(Fcμ) obtained by cleavage of the whole μ chain with CNBr at Met-225 (*19*).

The sequence from Val-211 through Met-225 is identical in two IgM proteins (Ou and Di), and the carbohydrate prosthetic group of the glycopeptide has the same composition (*20*). This, together with the apparent identity of peptide μ2 from different IgM proteins, confirms that the sequence shown in Fig. 1 is from the constant part of the μ chain. Oligosaccharides are located at four other sites on the constant part of the μ chain (*20*).

In the hinge region, the μ chain has little homology in sequence to the γ chains. In Fig. 1, of the more than 30 residues common to the two γ chains, only 8 are shared by the μ chain. The characteristic proline-rich sequence near the heavy-heavy interchain bridge of the γ chains is missing in the μ chain. Indeed, of the many peptides that we have isolated representing sequence around 14 different half-cystine residues in the entire μ chain, none has a high proline content.

In this region, which is characterized by its susceptibility to limited proteolytic cleavage, the μ chain has only one disulfide bridge compared to two in the rabbit γ chain, three in the human γ1 chain, four in the human γ2 chain, and up to five in the human γ3 chain (*21*). Because this region is high-

ly variable in all the heavy chains, gaps must be placed to obtain greatest homology. This procedure aligns the heavy-heavy interchain bridge and brings into focus the methionine-threonine substitution preceding it. The carbohydrate on this threonine in some rabbit γ chains (*8*) has a counterpart on the Asx residue (probably asparagine) that is located close to where the light-heavy interchain bridge occurs in the human γ1 chain and where an extra intrachain bridge is present in the rabbit γ chain. The large oligosaccharide on the μ chain would probably interfere sterically with the action of papain, just as the presence of carbohydrate on the threonine preceding the interchain bridge in the rabbit γ chain shifts the site of papain cleavage three to five residues in either direction (*8*). The failure of pepsin to form both Fab and Fc fragments in good yield from IgM may be related to the absence of the pepsin-sensitive sequence Leu-Leu and its replacement by the acidic sequence Asp-Glu-Asp, which would be very resistant to the enzyme.

The sites of tryptic cleavage differ in the three chains because of local differences in sequence and conformation. Although secondary cleavage may occur at basic residues preceding it, Arg-214 is the primary locus of tryptic cleavage at 60°C in the human μ chain. This site is not susceptible in the rabbit γ chain because of the presence of threonine, nor is it susceptible in the human γ1 chain despite the arginine-lysine interchange. Residue Lys-222, the site of tryptic cleavage in the human γ1 chain is not susceptible in the rabbit γ chain because it is followed by proline, nor in the human μ chain where it is replaced by alanine. At 37°C, Arg-239 appears to be the trypsin-sensitive residue in the μ chain, but it is replaced by serine in both γ chains. Trypsin cleaves the rabbit γ chain at Cys-220 after disruption of the disulfide bridge by conversion to the basic residue aminoethylcysteine (*22*). Trypsin does not form Fab and Fc fragments from intact rabbit IgG, perhaps because most basic residues in the hinge region are followed by proline or are flanked by proline and aspartic acid. An exception is Lys-213, which may be shielded by the extra intrachain bridge.

The above interpretation is supported by molecular models that we have made, which demonstrate a severe rigid distortion of the γ chains in the Cys-Pro-Pro area. Proline is unique among amino acids in being incompatible with the α-helix, and it gives to the sites where it occurs the least conformational freedom. Molecular models of the

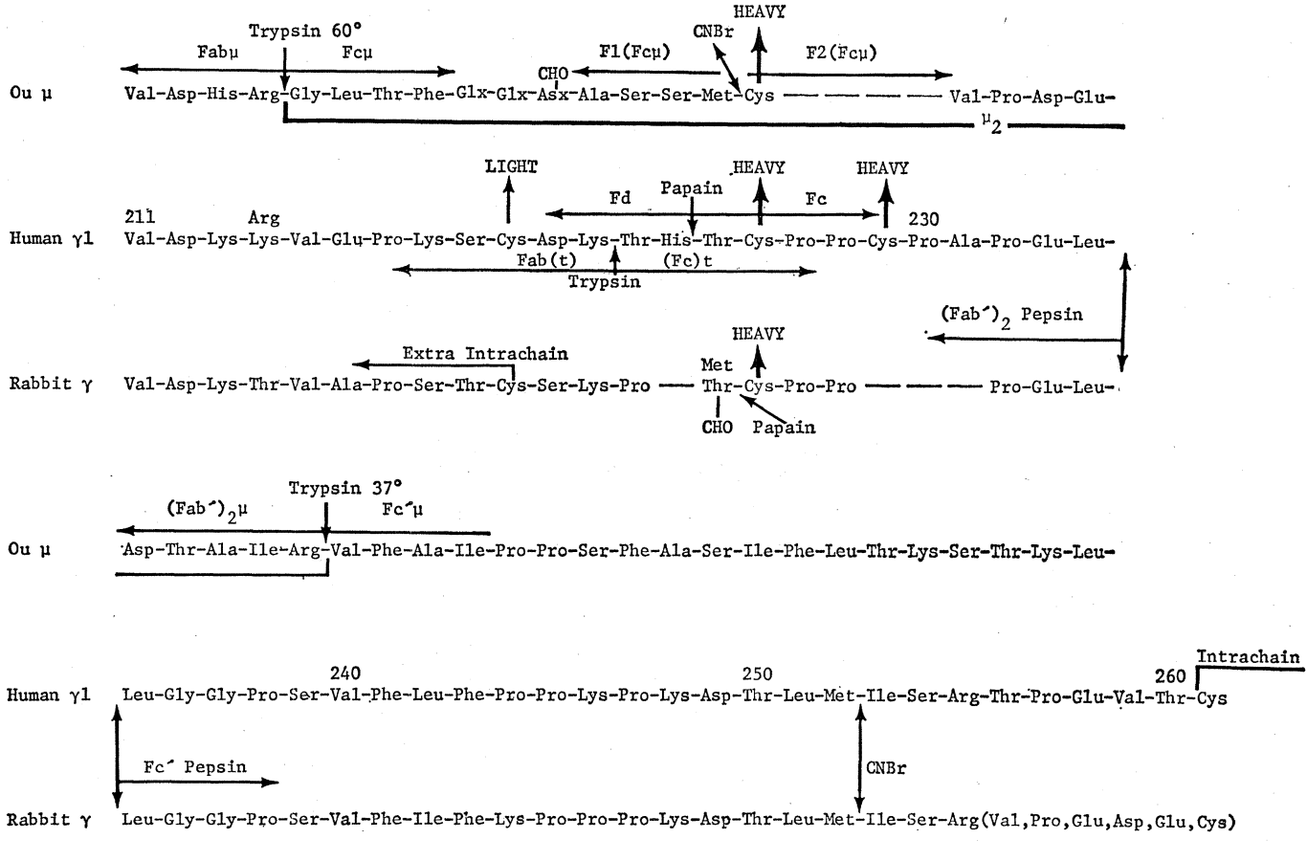

Fig. 1. Amino acid sequence in the hinge region of the μ chain from the human IgM globulin Ou and of the human γ1 and the rabbit γ chains. The sites of specific cleavage into Fab and Fc fragments by trypsin, papain, and pepsin are shown, and also the location of carbohydrate (CHO). CNBr cleaves the μ chain at the site designated to yield the fragments F1(Fcμ) and F2(Fcμ). The peptide μ2, containing the heavy-heavy interchain bridge, is present in (Fab')$_2\mu$ prepared by tryptic digestion at 37°C (*17*) but absent in Fabμ prepared at 60°C. The numbering system is for the human γ1 chain (*6, 7*). (See the text for discussion of the numbering of the μ chain.) Sequence data are from various sources for the human γ1 chain (*2, 6, 7*) and the rabbit γ chain (*8, 22*). Fab and Fc are the fragments of human and rabbit IgG prepared by incubation with papain at 37°C (*2, 24*). Fragment (Fab')$_2$ is the analogous dimeric fragment obtained with pepsin (*24*). Fabμ and Fcμ are here defined as the fragments obtained by tryptic cleavage of human IgM at 60°C (*9*) at the site indicated above, whereas (Fab')$_{2\mu}$ is the analogous dimeric fragment obtained with trypsin at 37°C where Fc is largely degraded to peptides (*3, 5, 17*). Fab comprises the intact light chain disulfide-bonded to the Fd or NH_2-terminal portion of a heavy chain. Fc represents the COOH-terminal portion of a heavy chain and may be a monomer or dimer (IgG) or a decamer (IgM) depending on the state of the heavy-heavy interchain bridges.

hinge region of the human γ1 chain show that, when a number of prolines occur as in the sequence Cys-Pro-Pro-Cys-Pro-Ala-Pro and when two such sequences are joined by a pair of disulfide bonds, a severe rigid distortion occurs. This interrupts the conformational folding in the middle of the heavy chain between the second and the third intrachain disulfide loops, thereby imparting a looseness or extended chain conformation that leads to an apparent flexibility in the structure (*23*). Proline thus provides the rigid part of a flexible hinge. A distortion due to proline is absent in the μ chain. However, it may be provided by the presence of the large, hydrophilic oligosaccharide which requires that the hinge region must be at the surface of the IgM pentamer. Incubation at 60°C may partially separate the monomeric IgM subunits or the two μ chains within the subunit, thereby making the Arg-Gly bond accessible to trypsin. The temperature effect is probably on the conformation of the immunoglobulin, rather than on the trypsin, for we find that incubation with thermolysin at 60°C, but not at 37°C, produces similar Fabμ and Fcμ pieces. Within each subunit the Fcμ dimer is linked by two interchain disulfide bridges, one near the NH_2-terminus and one at the COOH-terminus. The subunits are linked by the intersubunit bridge, which is not within the hinge region depicted in Fig. 1.

Although papain often cleaves μ and γ chains at multiple sites to give heterogeneous Fc fragments (*2, 24*), trypsin, which is a more specific enzyme, acts predominantly at a single site on the μ chain to produce an apparently homogeneous Fcμ piece. This is confirmed by the behavior of Fcμ in immunoelectrophoresis, immunodiffusion, and ultracentrifugation, and also by the finding of a unique amino acid sequence with the sequencer. The specific enzymatic and chemical cleavage of IgM and the combined use of the sequencer and of conventional sequencing techniques have greatly facilitated the determination of the amino acid sequence of this protein, which has a covalent molecular weight of about 1 million. The same principles can be applied to other large molecules that have resisted the conventional methods of sequence analysis.

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Vitamin A Deficiency Effect on Retina: Dependence on Light

Abstract. *The effects of vitamin A deficiency in the rat eye, as measured by the electroretinogram and changes in rhodopsin content, are critically dependent upon the levels of illumination to which the animals are exposed daily. Depleted animals kept in darkness maintained virtually normal electroretinogram function and rhodopsin content for 5 to 6 months while those kept in weak cyclic light lost rhodopsin continuously. A fraction of the retinol released from rhodopsin during illumination disappears presumably from the pigment epithelium into the blood and becomes unavailable for rhodopsin regeneration. A sequence of three first-order reactions was assumed to estimate the rate constant of this disappearance (0.03 per hour). Computer simulation supporting the experimental data illustrates the dependence of the retinal abnormalities on light.*

Dowling and Wald have analyzed the effect of nutritional vitamin A deficiency upon the retina of the albino rat (*1*). Night blindness, the first sign of tissue deprivation, measured by the rise in the threshold for eliciting the electroretinogram (ERG), was directly related to the loss of vitamin A aldehyde from the visual pigment. The effect appeared as early as 3½ weeks on the dietary regimen and progressed inevitably to the almost complete disappearance of electrical responsiveness. Structural deterioration of the outer rod segment occurred in conjunction with a decline of the protein component of the visual pigment, and a "substantial fraction" of the visual cell population seemed to undergo lysis and disappear. Treatment with retinoic acid, while relieving the systemic effects of the deficiency, had no influence on the progress of the retinal abnormality but greatly facilitated the study of the advanced stages of the disease.

We reinvestigated the effects of vita-