magnitude: palmitic, myristic, lauric, oleic, and linoleic; they also comprised 87 percent of the total. The results indicated that the relative concentrations of the shorter-chain fatty acids in the tumor fluid were higher than those of milk. A recent finding by Rees et al. was similar (8); they analyzed the fatty acids in the dimethylbenzanthracene-induced mammary carcinomas of animals being treated with estrogen, finding that the lipids from those neoplasms had a higher proportion of C₁₀-C₁₄ fatty acids than had milk.

One of the most characteristic components of milk is casein, and its presence in milk and tumor fluid was determined. Whole rat milk was treated with acetate buffer, pH 4.6, to precipitate casein. The supernatant was collected, since it contains the whey proteins, and lyophilized. Both preparations were solubilized in 7M urea and subjected to starch-gel electrophoresis at pH 8.6 for 2 or 3 hours (9). The proteins were localized by staining with Ponceau S at 0.2 percent in a 3-percent solution of trichloroacetic acid. The tumor fluid was handled similarly, and both fluids in one cup were simultaneously subjected to electrophoresis (Fig. 1). A protein component in the tumor fluid proved similar in electrophoretic mobility to casein from rat milk, and two protein components in the whey fraction of the tumor fluid apparently showed electrophoretic properties similar to those of the whey proteins of rat milk. One should note that there were many more protein components in the tumor fluid than in milk.

These studies indicate that the milklike accumulation in the R3230AC adenocarcinoma after treatment with estrogen contained several substances that occur in rat milk. I should point out that quantitative measurements of these components in the tumor fluid are subject to error, since the tumor fluid undoubtedly contained tissue and cell components-contaminants that could not be completely avoided because of the lack of a duct system in the neoplasm and because of the method used for collecting this fluid. However, my data demonstrate that this adenocarcinoma of the breast can produce a milk-like fluid under the influence of estrogen.

RUSSELL HILF

Squibb Institute for Medical Research, New Brunswick, New Jersey

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Visual Accommodation in the Green Turtle

Abstract. Ophthalmological and anatomical studies indicate that the Atlantic green turtle is extremely myopic when its eyes are out of water. This finding bears on current theories of visual orientation and navigation in these animals.

Celestial navigation has been considered as a possible guidance mechanism underlying the impressive homing feats of the Atlantic green turtle (Chelonia mydas) (1), yet nothing is known about the visual acuity of these marine animals. The freshwater turtles, because they have exceptionally welldeveloped ciliary and iris musculature and a highly flexible lens, are able to accommodate over a wide dioptric range, and they have a high visual acuity in both air and water (2). However, the loggerhead turtle, Caretta (Thalassochelys) caretta, the only marine turtle whose vision has been examined, was found by Beer (3) to be highly myopic on land; this finding was confirmed by Walls (4).

Errors in refraction in two immature green turtles, weighing 1 kg and 17 kg, were measured retinoscopically. Measurements were made in air and also while the turtles were under water in a flat-sided, glass aquarium. For purposes of comparison, a freshwater turtle (Clemmys insculpta) and two land tortoises (Gopherus polyphemus) were also examined (Table 1).

The green turtles were approximately

Table 1. Refractive errors (in diopters) of three species of turtle measured in air and water.

| | Air | Water |
|---------------------|------|--------------|
| Chelonia mydas | 40 | 0 |
| · . | 40 | 0 |
| Clemmys insculpta | 0 | -1 |
| Gopherus polyphemus | -5.5 | -45 to -50 |
| | -5.5 | -45 to -50 |

emmetropic when submerged in an aquarium, but extremely myopic while in air. In contrast, the tortoises were somewhat hyperopic in air and, like humans, were unable to overcome the loss of corneal refraction under water. As previously reported in the literature, the freshwater turtle adapted rapidly to both media.

Gross and microscopic examination of sections of the eyes of an adult green turtle and the eyes of a member of another species of marine turtle, the hawksbill (Eretmocheyls imbricata), extended and supported the conclusions of Walls (4) that marine turtles lack some of the efficient mechanisms of accommodation found in freshwater turtles. The ciliary processes did not impinge directly upon the body of the lens, and there was no apparent expansion of the sphincter iridis muscle into a powerful muscle of accommodation.

Except when surfacing to breathe and possibly to orient, the only time green turtles may need aerial vision is when the mature females go ashore to nest and when the hatchlings emerge from the nest and head for the ocean. Ehrenfeld and Carr (5) studied the sea-finding orientation of the green turtle and found this to be largely a visual process. They observed, however, no significant reduction in the ability of mature females to find the sea when they wore spectacles that contained light-diffusing filters. This is not surprising, since the retinoscopic measurements and anatomical evidence indicate that these animals could hardly have been using an orientation mechanism that depended upon the formation of sharp retinal images when on land.

The implications of the green turtle's limited visual acuity in air also extend to considerations of guidance mechanisms during their lengthy, open-sea migrations. Bi-coordinate star navigation no longer seems to be a realistic hypothesis. It is extremely unlikely that turtles can perceive stars or star configurations when their heads are above water. Although it is theoretically possible for them to see stars from beneath the surface, this would only occur in perfectly calm water. The normal ocean waves would disrupt the star images and, because of the critical angle of refraction, would impose a new and shifting horizon. Other types of celestial navigation involving the position of the sun or moon cannot be ruled out; but any theories must be consistent with knowledge of the green turtle's inability to see clearly outside its marine environment.

> DAVID W. EHRENFELD ARTHUR L. KOCH

Departments of Zoology and Biochemistry, University of Florida, Gainesville 32601

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- We thank Dr. Richard Copenhaver for per-forming the retinoscopic examinations, and E. Gourley, Dr. C. I. Hood, and Mrs. B. O'Conner for advice and assistance. The work was carried out while D.W.E. was a fellow of NIH (5-F3-GM-20, 196-02), and it was sup-ported by NSF and ONR grants GB-3910 and NONE 580 (12) accordingly (A Corr Tria NONR-580 (12), respectively (A. Carr, prin-cipal investigator), and by the Caribbean Conservation Corporation.
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Evolution of Immunoglobulins: Structural Homology of Kappa and Lambda Bence Jones Proteins

Abstract. The amino acid sequence of a human lamba chain has been determined. There are many identities in sequence with human kappa chains, but this intraspecies homology is less than the interspecies homology of kappa light chains of man and mouse. Structural relationships suggest a common evolutionary origin and early differentiation of light- and heavy-chain genes.

Immunoglobulin molecules consist of a pair of heavy chains that determine that γ -globulin class (γG , γA , or γM) and a pair of light chains (kappa or lambda) that determine the antigenic type (K or L, respectively) (1). Patients with multiple myeloma excrete Bence Jones proteins identical in structure and antigenic type with the light chains of the myeloma globulin in their serum and similar to the light chains of nor-

mal γ -globulin (2). Comparison of amino acid sequences of type K Bence Jones proteins of man (3, 4) and the mouse (5) has established that the polypeptide chains consist of a variable portion and an invariant portion. In man the invariant portion comprises the 107 amino acids in the COOH-terminal end, except that residue 191 is leucine in molecules of the genetic type $Inv(a^+)$ and valine in the $Inv(b^+)$ type (6). The NH₂-terminal portion (also about 107 residues) is subject to variation; up to 23 positions have been found to be substituted when limited sequence areas of some half dozen human Bence Jones proteins of type K have been compared (7) and 42 positions in two mouse Bence Jones proteins of type K (5). Thus, the hypothesis has been proposed that all light chains (and probably also heavy chains) contain a variable and an invariant region, the amino acid sequence of which is related to antibody specificity (3, 4).

Although the two antigenic types of human Bence Jones proteins and of the light chains of immunoglobulins (types K and L) share no antigenic determinants and have no tryptic peptides in common (8), it has been predicted that they should exhibit considerable homology in primary structure (7, 8). This prediction has now been verified by analysis of amino acid sequence of a human Bence Jones protein of type L. Between one protein of type K (designated Ag) for which the tentative complete sequence is known (3, 7) and one protein of type L (designated Sh) for which the probable sequence is given in Fig. 1, there are many positions of identity or probable identity and many others where the amino acid pairs are chemically homologous or are related to the genetic code through single nucleotide changes within single codons.

The type L Bence Jones protein designated Sh was purified on a Sephadex A-50 column. The protein was reduced with mercaptoethanol in 7M guanidine hydrochloride; the reduced protein was then aminoethylated by reaction with ethylenimine for subsequent tryptic digestion (9), or it was alkylated with monoiodoacetic acid for subsequent chymotryptic digestion. The peptides of the tryptic digest were first fractionated on a Dowex-1-X2 column by use of a gradient system with volatile buffers (4). The peptide fractions thus obtained were purified on Dowex-50-X2 columns by use of pyridine-acetic acid buffers. Altogether, 21 major tryptic peptides were characterized. Two of them contained two basic amino acids: one contained one lysine and one arginine, the other contained two arginines. Amino acid analysis on the intact protein indicated that all the basic amino acids (11 lysine, 7 arginine, and 5 aminoethylcysteine residues) were recovered in the tryptic peptides.

In a separate experiment, the chymotryptic digest of the S-carboxymethylated protein was placed on a Dowex-1-X2 column, and the peptides were eluted with pyridine-acetate buffers graded with respect to pH and ionic strength. Dowex-50-X2 columns were used for further purification of the peptide fractions, as well as for the tryptic peptides. A sufficient number of peptides was isolated to permit us to propose a unique arrangement of the tryptic peptides in protein Sh. In some instances, the overlapping chymotryptic peptides were digested with trypsin in order to confirm the assignment of linkages between tryptic peptides. The amino acid sequence of the tryptic and chymotryptic peptides was determined by stepwise degradation by the modified Edman method (10) and by use of leucine aminopeptidase and carboxypeptidase A.

Because of previous difficulty in determining the NH2-terminal amino acid of type L Bence Jones proteins (8, 11), special attention was given to the assignment of the NH₂- and COOH-terminal residues. Serine was obtained as the NH₂-terminal residue when the dinitrophenyl method was applied to the intact protein, but in low yield. Also, serine is the NH2-terminal residue of the chymotryptic peptide which has the same starting sequence as the tryptic peptide given as NH₂-terminal in Fig. 1 (the initial octadecapeptide ending in arginine). The sequence of the octapeptide shown as COOH-terminal in Fig. 1 confirms that given by Milstein (12) for γG light chains of type L and accords with the composition of the COOH-terminal peptide A1 reported by Putnam and Easley in all type L Bence Jones proteins they studied (8).

Because protein Ag contains 214 amino acids according to sequence analysis, the previous numbering system based on the assumed 212 residues in the Roy protein has been changed (13). Although the Sh protein appears to have 213 residues, a numbering system is not proposed yet since the proposal for the sequence is still tentative. However, when the sequences