$C_{11}H_{12}N_2O$ for compound A and $C_{10}H_{10}N_2O$ for compound B (Table 1) (*4*). The facile loss of C_2H_4, HCN, and CO from the former, and of CH_3CN and CO from the latter, considered along with the other spectral data, led us to assign the 1,2-dialkyl-4(3*H*)-quinazolinone structures I and II to A and B, respectively.

A search of the literature showed that both I and II had been synthesized and carefully described in 1961 (*5*). Their published infrared and ultraviolet spectra, as well as their melting points, are in excellent agreement with those of A and B, thus completing the identification.

While our studies on *G. marginata* were in progress, Schildknecht *et al.* (*6*) described the isolation from the same millipede secretion of a crystalline compound which they named "glomerin," for which no structure was deduced. The properties of "glomerin" correspond closely to those of our substance B, and therefore "glomerin" is established to be 1,2-dimethyl-4(3*H*)-quinazolinone (II).

Besides the two heterocyclic compounds, the *Glomeris* secretion must contain other, possibly macromolecular, components. When first discharged, the secretion is translucent and liquid, but within seconds after exposure to air it becomes viscous and sticky, and eventually hardens to an opaque crust. The two identified constituents cannot, by themselves, account for these changes.

Various plant alkaloids are based on the quinazoline ring system (III) (*7*). Both natural and synthetic quinazolines possess significant biological activities, including antimalarial activity (*7*). The most closely related natural product appears to be arborine (IV), the chief alkaloid of the Indian medicinal plant *Glycomis arborea* Correa, in which the propionate and acetate moieties in I and II are replaced by a phenylacetate moiety (*5*). We have recently reported the characterization of a pyrrolizidinone, closely related to the Senecio alkaloids, from the hair-pencil secretion of a danaid butterfly (*8*). The finding of I and II in a millipede secretion now provides the second example of close structural parallelism between an arthropod product and the plant alkaloids.

The defensive behavior of *Glomeris*, the repellent effectiveness of its secretion, and the morphology of the glands have also been studied (*9*).

Note added in proof. Since the submission of this manuscript, a more recent publication of Schildknecht and Wenneis, in which "glomerin" is assigned structure II on the basis of independent evidence, has come to hand (*10*).

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24 August 1966

Light Chains of Mouse Myeloma Proteins: Partial Amino Acid Sequence

Abstract. *Five kappa chains in the urinary proteins of the BALB/c mouse have the same carboxyl terminal amino acid sequence; this sequence resembles that of kappa light chains in human immunoglobulins. The five chains have amino acid sequence variations at the amino-terminal. The genetic basis for the amino-terminal variations is not understood but could be due either to a mechanism for differently translating a single genetic message or to the presence of more than one kappa-type structural cistron in the BALB/c genome.*

The genetic basis of the heterogeneity of the light and heavy chains of related immunoglobulins poses the question of whether the polypeptide chain variants are a consequence of a large number of genes or a consequence of mechanisms for varying the protein product of a single gene by somatic mutation (chemical mutagenesis, crossing over, and others); or, alternatively, whether there is a versatile mechanism for translating a single genetic message (*1*). In an attempt to solve this problem we have investigated the amino acid sequence of related variant polypeptide chains that have originated in genetically similar animals, using the kappa-chain urinary proteins of the inbred BALB/c mouse (*2*) as an experimental model. Partial amino acid sequences of several kappa chains in the urinary proteins of patients with multiple myeloma have been reported (*3*), and we present partial amino acid sequences of several kappa chains in the urinary proteins of mice.

Kappa-chain (*4*) urinary proteins produced by plasma-cell tumors MOPC 70E, MOPC 41, MOPC 157, MOPC 46, and Adj-PC-9 from the highly inbred BALB/c strain of mice were used (*5*). Carboxymethylated preparations of these proteins were electrophoretically homogeneous in polyacrylamide gels, except for the protein MOPC 46, which consistently showed four to five bands.

The NH_2-terminal analyses were performed by the 2,4-dinitrofluorobenzene method (*6*). Phenylisothiocyanate degradation of the proteins was performed by the paper-strip procedure (*6*). Identification of the phenyl-

Table 1. Dinitrophenyl-peptides (S_1, S_2, and S_3) isolated from kappa chains digested with subtilopeptidase A.

Protein	Peptides		
	S_1	S_2	S_3
MOPC-70E	DNP-Asp-(Ile, Val)	DNP-Asp-(Ile, Val, Leu)	DNP-Asp-(Ile, Val, Leu, Thr)
MOPC-41	DNP-Asp-(Ile, Gln)		
MOPC-46	DNP-Asp-(Ile, Val)	DNP-Asp-(Ile, Val, Leu)	
MOPC-157	DNP-Asp-(Ile, Val)		

Table 2. Composition of COOH-terminal peptides isolated from tryptic digest of kappa chains oxidized with performic acid.

Protein	Peptide composition (μmole)		
	$CySO_3H$	Glu	Asp
MOPC-70E	0.094	0.080	0.084
MOPC-41	.095	.089	.086
MOPC-157	.103	.104	.103
MOPC-46	.101	.096	.097
Adj-PC-9	.103	.097	.096

thiohydantoin residue released was based on chromatographic analysis in the solvent system of Edman and Sjoquist (*7*) and by regeneration of the amino acid by hydrolysis in 6*N* HCl at 150°C for 20 hours. For NH_2-terminal analysis the proteins were also coupled with DNS-Cl (*8*) at 37°C, and hydrolyzed with 6*N* HCl, and the resulting DNS-amino acid was identified by high-voltage electrophoresis on paper in parallel with mixtures of standard DNS-amino acids for comparison (*9*).

The DNP-peptides were isolated after hydrolysis of the DNP-proteins by subtilopeptidase A (subtilisin). The hydrolyzate was freeze-dried and dissolved in 1*N* HCl, and the DNP-peptides were extracted with ethyl acetate, washed once with water, and concentrated on a rotary evaporator at 30°C. The DNP-peptides were further purified by successive electrophoresis on paper at *p*H 6.5 (in a mixture of pyridine and acetic acid) and at *p*H 8.9 (in 1 percent ammonium carbonate). The NH_2-terminal peptide of the protein MOPC 41 was also isolated after treatment of the protein with cyanogen bromide in 20 percent formic acid (*10*). The NH_2-terminal peptide was recovered by gel filtration on Sephadex G-25. The protein from tumor Adj-PC-9 was digested with subtilopeptidase A at *p*H 8.0, and peptide material lacking a free α-amino group was separated from all other peptides on an ion exchange (Dowex 50 X2-H+) column and further fractionated by high-voltage paper electrophoresis. For COOH-terminal analysis, the samples were oxidized with performic acid (*11*) and subjected to hydrazinolysis in sealed, evacuated tubes (*12*). For isolation of COOH-terminal peptides high-voltage electrophoresis on paper at *p*H 6.5 and *p*H 3.6 was used after digestion of the proteins with trypsin. The peptides were degraded by a modification (*13*) of the Edman method.

End groups of the proteins were analyzed in several different experiments. By the DNP method, aspartic acid (or asparagine) was shown to be the NH_2-terminal amino acid in each case except in the Adj-PC-9 protein, which consistently failed to reveal a free NH_2-terminal group. By the phenylisothiocyanate method in each case (Adj-PC-9 excepted) a phenylthiohydantoin derivative having the same R_F value as the phenylthiohydantoin of aspartic acid (distinguished from that of asparagine) was recovered. Estimation of the phenylthiohydantoin of aspartic acid by spectroscopy showed that there was 0.8 to 1.0 residue of NH_2-terminal aspartic acid (uncorrected for losses) per 25,000 g of protein. The phenylthiohydantoin residues released in the second step of the method were isoleucine (or leucine), valine, and glycine, which suggests that some random cleavage of internal peptide bonds had occurred during the cyclization procedure. Again Adj-PC-9 protein showed no free NH_2-terminal group. The results of coupling with DNS-Cl confirmed those obtained by the other methods with all the proteins.

On the basis of analyses of the DNP-peptides (Table 1) the terminal sequences NH_2-Asp-(Ile, Val)-Leu-Thr and NH_2-Asp-(Ile, Val)-Leu can be assigned to MOPC 70E and MOPC 46 respectively. The amino-terminal sequence of MOPC 157 is NH_2-Asp-(Ile, Val), whereas MOPC 41 has the sequence NH_2-Asp-(Ile, Gln). This result for MOPC 41 has been confirmed and extended by the isolation of the NH_2-terminal tetrapeptide NH_2-Asp-Ile-Gln-HoSer after cyanogen bromide cleavage of the protein. The peptide (Glu, Leu, Ile, Val) was found in the water eluate from a Dowex (50 X2-H+) column to which the subtilisin digest of Adj-PC-9 protein had been applied. The peptide was ninhydrin-negative and lacked a free NH_2-terminal amino acid, and its electrophoretic mobility at *p*H 6.5 indicated a single negative charge at this *p*H. This, together with the fact that Adj-PC-9 protein also lacks a free NH_2-terminal amino acid, indicates that this protein has the NH_2-terminal sequence Glu-(Ile, Val, Leu), with the glutamic acid in the form of the pyrrolidonecarboxylic acid [see Porter and Press (*14*)].

Carboxyl-terminal analysis of the proteins, oxidized with performic acid, by the hydrazinolysis method, showed only cysteic acid in significant amounts when digests were chromatographed on the amino acid analyzer. One peptide which contained neither arginine nor lysine was obtained in good yield from tryptic digests of all five kappa chains oxidized with performic acid. Table 2 shows the amino acid composition of the COOH-terminal peptides; and the COOH-terminal sequence was established as (Lys or Arg)-Asn-Glu-Cys in all five proteins by Edman degradation.

Five kappa chains orginating in genetically similar mice are closely related structurally, having common tryptic peptides and antigenic determinants (*2*), and a common carboxyl terminal amino acid sequence for at least the last three residues. Compositional analysis (*15*) reveals many amino acid differences in these proteins, and some were found in the NH_2-terminal sequence.

A comparison of the COOH-terminal sequence of the mouse and human kappa chains (Table 3) indicates homology between the two species and suggests that the genes controlling these chains originated from a common ancestral gene. On this basis further homologies in the mammalian proteins may be expected (for example, the NH_2-terminal sequences of MOPC 41 and Ag protein are identical, Table 3). The carboxyl terminal cysteine of human kappa light chains forms an interchain disulfide bond with the heavy chain (*16*). The selective union of the light and heavy chains by this specific

Table 3. Amino acid sequence of kappa chains.

Protein	NH_2-terminal	COOH-terminal
MOPC-70E	Asp-(Ile, Val)-Leu-Thr	Asn-Glu-Cys
MOPC-41 (*17*)	Asp-Ile-Gln-Met	Asn-Glu-Cys
MOPC-46	Asp-(Ile, Val)-Leu	*Ser-Phe-Asn-Arg-Asn-Glu-Cys
MOPC-157	Asp-(Ile, Val)	Asn-Glu-Cys
Adj-PC-9	†PCA-(Ile, Val, Leu)	Asn-Glu-Cys
Human Ag (*3*)	Asp-Ile-Gln-Met-Thr	Ser-Phe-Asn-Arg-Gly-Glu-Cys

* The additional amino acid sequence information given here is derived from peptides isolated from the peptic and tryptic digests of performic-acid-oxidized MOPC-46 (R. N. Perham, unpublished observations). † Pyrrolidonecarboxylic acid.

bond may possibly be regulated by the neighboring amino acid sequence, thereby explaining the preservation of this sequence in the different species. The mechanism for variations in the amino terminal sequence cannot be explained by our data. Perhaps there is more than one kappa chain cistron resulting from reduplication. Independently occurring mutations in several of these cistrons could be transmitted in the germ line and account for the observed amino acid differences. Regarding stability and economy of genetic material one might ask if some mechanism allows a common genetic message to be translated into different polypeptide chain products (*1*).

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Protein-Polysaccharide Loss during Endochondral Ossification: Immunochemical Evidence

Abstract. *Fluorescein-labeled antibodies to protein-polysaccharides from rat and calf cartilage were used for the histochemical localization of protein-polysaccharide in epiphyseal cartilage. There was less protein-polysaccharide in the zone of provisional calcification than in the proliferating and maturing zones, and none was demonstrable in the metaphysis. During or just preceding calcification, protein-polysaccharide or its protein component is lost or drastically altered.*

The anionic polysaccharides of cartilage are, for the most part, covalently linked to protein other than collagen (*1*); and they can be extracted with water from cartilages by high-speed homogenization and subsequently separated by differential centrifugation into, at least, a heavy fraction (PP-H) and a light fraction (PP-L). From its behavior in the ultracentrifuge, the light fraction may be relatively homogeneous, although polydisperse (*2, 3*).

During endochondral ossification, much of the polysaccharide is eliminated; analytical and autoradiographic data (*4, 5*) indicate that only 20 to 25 percent of the sulfate in the cartilage persists in the metaphysis. This significant change in sulfate concentration of the organic matrix may be related to the process of calcification (*4, 6*). In large measure, the sulfate in the metaphysis is part of material akin to chondroitin sulfate (*7*). Histological evidence of changes in the metachromatic properites of cartilage matrix in the hypertrophic zone (*8*) also suggests an alteration in the nature or state of combination of the mucopolysaccharides related to calcification. Extraction of protein-polysaccharides resembling those in cartilage has been unsuccessful when techniques applicable to cartilage were used on fresh or decalcified samples of metaphyseal bone (*9*). Possibly, in the process of endochondral ossification the protein in the protein-polysaccharides may be modified or eliminated. Indeed, there is a protease in cartilages which, in vitro, snips away about 25 percent of the protein in PP-L. This product is much more soluble than the PP-L from which it is derived. Conceivably, such a modification of PP-L may be an essential step for its elimination from cartilage or for further modifications leading to its partial retention in the developing metaphyseal trabeculae.

Precipitating antibodies have not been produced in animals injected with chondroitin sulfate or hyaluronate (*10*), but precipitating antibodies against PP-L from various cartilages (*11, 12*) and against hyaluronate-protein (*13*) have been produced in rabbits and guinea pigs. Therefore, the associated proteins seem to be necessary for antigenicity. The precipitin reaction of PP-L or of hyaluronate-protein with its antiserum is eliminated by prior treatment of the antigen with some proteolytic enzymes, but the reaction with antiserum proceeds after similar treatment with testicular hyaluronidase (*12–14*).

Accordingly, we have used the fluorescent antibody technique to test whether the concentration of PP-L decreases concomitantly with the calcification of the matrix of growth cartilages, and whether PP-L is present in the metaphyses.

Protein-polysaccharides were extracted into water by high-speed homogenization of costal cartilage of calves and of epiphyseal cartilage of young rats (12 to 14 days old) (*15*) and further purified (*2*). The sample of PP-L from the costal cartilage of calves decreased in weight by 12.11 percent

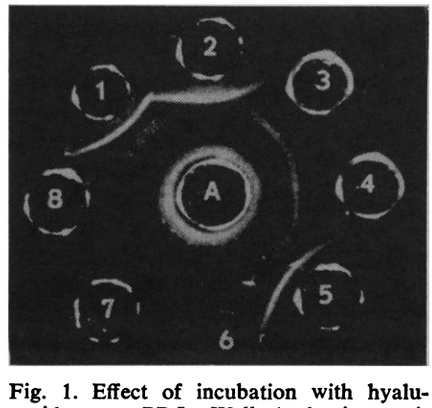

Fig. 1. Effect of incubation with hyaluronidase on PP-L. Well A, bovine antiserum to PP-L; wells 1 and 5, bovine PP-L incubated with hyaluronidase; well 2, bovine PP-L; well 3, rat PP-L; well 4, rat PP-L incubated with hyaluronidase; well 6, hyaluronidase alone; wells 7 and 8 not used.