Polypeptide Chains of Immunoglobulins from the Smooth Dogfish (Mustelus canis)

Abstract. *The 17S and 7S immunoglobulins of the smooth dogfish,* Mustelus canis, *have been characterized with respect to molecular weights, chain structure, amino acid composition, and carbohydrate content. The 17S protein had a molecular weight of 982,000; that of the 7S protein was 198,000. Both proteins had similar amino acid compositions and a carbohydrate content of approximately 8 percent. Light and heavy chains from both immunoglobulins had molecular weights of 20,000 and 72,000 respectively. Apparently both immunoglobulins belong to the same class; this class resembles the γM-immunoglobulins of higher vertebrates.*

The elasmobranch *Mustelus canis* has immunoglobulins with sedimentation coefficients of approximately 7*S* and 17*S* (*1*). These immunoglobulins are composed of light and heavy polypeptide chains. The heavy chains of both immunoglobulins had similar peptide maps and they resembled the μ-chains (*1, 2*) of higher vertebrates when acquired by starch-gel electrophoresis. It was therefore suggested that *M. canis* may have only one major class of immunoglobulins resembling that of the γM-immunoglobulins seen in higher vertebrates. We now report the molecular weights, carbohydrate contents, and amino acid compositions of dogfish immunoglobulins and their polypeptide chains. Our results strengthen the concept that these immunoglobulins are related to the γM class described in higher vertebrates.

A direct comparison of the chains of dogfish 7*S* and 17*S* immunoglobulins with those of human γG- and γM-immunoglobulins is shown (Fig. 1). The immunoglobulins were reduced and alkylated in 8*M* urea (*1*) and were subjected to starch-gel electrophoresis in urea (*3*). The light chains of all of the samples had similar mobilities. The heavy chains of both the 7*S* and 17*S* immunoglobulins of the dogfish had mobilities similar to that of μ-chains from the human γM-immunoglobulin (compare Fig. 1, origins 2, 3, and 4).

Carbohydrate analyses of the dogfish immunoglobulins were carried out with anthrone reagent (*4*). The 7*S* immunoglobulin contained 7.6 ± 1.1 percent of carbohydrate (by weight) and the 17*S* immunoglobulin contained 8.7 ± 0.9 percent. These values are within the range observed for mammalian γM-immunoglobulins (*5*). The γM- and γA-immunoglobulins cannot be distinguished by this criterion alone, inasmuch as γA-immunoglobulins may contain 6 to 10 percent carbohydrate (*6*).

Amino acid compositions (*7*) of dogfish immunoglobulins and their chains are presented in Table 1. The dogfish immunoglobulins resemble human immunoglobulins in their high content of serine, threonine, aspartic acid, and glutamic acid. The heavy chains of the 7*S* and 17*S* immunoglobulins have similar compositions, and their compositions more nearly resemble those of human μ-chains than of human γ-chains. Light chains of the two dogfish immunoglobulins were also similar in composition, which differed significantly from that of the heavy chains, as well as from that of human light chains.

On the basis of the relative amounts of heavy and light chains in the dogfish immunoglobulins, it was suggested that the 7*S* protein consists of two light and two heavy chains and that the 17*S* protein contains five units, each similar to a 7*S* moiety (*1*). These structures would resemble those proposed for mammalian γM-immunoglobulins (*8, 9*). The molecular weights of dogfish immunoglobulins and their chains (Table 2) are consistent with this interpretation. The values were determined by the high-speed equilibrium method of Yphantis (*10*). Whole immunoglobulins were dissolved in 0.15*M* NaCl buffered to *p*H 8.0 with 0.05*M* tris. The chains, which were isolated after extensive reduction in urea (*1*), were dissolved either in 20 percent acetic acid containing 0.5 percent sucrose or in 1*M* propionic acid containing 0.5 percent sucrose. Partial specific volumes ($\bar{V}$) of the immunoglobulins and chains were calculated

Table 1. Comparison of amino acid compositions of immunoglobulins and polypeptide chains from *Mustelus canis* and man. Results are expressed as grams per 100 grams of carbohydrate-free protein. Cysteine and tryptophan values are not included. Variation in tyrosine values is probably due to oxidation during hydrolysis.

Amino acid	*Mustelus canis*						Man				
	Immunoglobulins		Chains				Immunoglobulins		Chains		
			Light		Heavy						
	17*S*	7*S*	17*S*	7*S*	17*S*	7*S*	γM*	γG*	Light†	γ-chains‡	μ-chains†
Lys	5.5	5.6	4.8	5.2	5.8	6.1	6.2	7.4	6.2	6.2	7.4
His	1.9	1.7	1.7	1.9	2.1	1.9	2.3	1.9	1.8	2.5	2.6
Arg	5.0	5.7	5.4	5.5	5.2	4.8	6.1	4.3	4.6	6.5	3.8
Asp	9.9	9.5	10.2	10.3	9.2	9.1	9.1	8.6	7.3	9.0	7.6
Thr	8.4	8.6	9.2	8.1	8.0	8.9	8.8	6.3	7.4	8.8	6.8
Ser	9.6	9.5	11.8	11.5	10.1	9.9	9.4	10.4	10.3	8.4	8.6
Glu	13.3	12.6	11.3	11.7	12.3	12.7	12.2	11.7	13.0	12.2	10.1
Pro	5.9	5.4	5.4	5.2	6.3	6.6	6.4	6.8	5.1	6.5	6.4
Gly	4.8	3.9	4.1	4.5	3.6	3.5	4.0	3.9	3.3	3.8	3.2
Ala	5.0	4.6	4.5	3.7	4.2	4.0	4.4	3.8	4.2	4.4	2.7
Val	7.1	7.4	7.0	7.6	7.1	7.8	7.7	8.5	6.3	8.1	8.2
Met	1.3	1.3	1.3	1.0	1.3	1.2	1.5	1.1	0.4	1.5	1.0
Ileu	5.3	5.6	4.5	4.7	5.8	6.4	2.7	2.3	2.7	3.4	1.9
Leu	7.7	8.0	8.5	8.2	7.4	7.9	8.1	8.3	7.3	8.1	7.1
Tyr	5.4	5.9	(6.3)	(6.9)	(7.4)	(4.3)	4.4	6.3	6.2	4.5	5.5
Phe	4.1	5.5	4.1	4.3	4.2	4.7	4.9	4.8	4.3	3.4	3.8

* Data of Heimer *et al.* (*14*). † Data of Crumpton and Wilkinson (*15*). ‡ Calculated from Chaplin *et al.* (*16*).

Table 2. Molecular weights of immunoglobulins and polypeptide chains from *Mustelus canis*. The molecular weights were determined by the high-speed equilibrium method of Yphantis (*10*). Molecular weights and standard deviations were calculated from a weighted least squares analysis as suggested by Yphantis (*10*).

Immunoglobulin	Molecular weights		
	Intact molecule	Light chain	Heavy chain
17*S*	982,000 ± 25,500	20,100 ± 500	71,600 ± 2,800
7*S*	198,000 ± 6,000	20,500 ± 300	73,400 ± 4,000

from their amino acid and carbohydrate contents.

On the assumption that no carbohydrate was present, the $\bar{V}$ of light chains was 0.727. The value obtained for heavy chains, if all of the carbohydrate was present on this portion of the molecule, was 0.723. Both whole immunoglobulins had values of 0.725, close to the value of 0.722 (*9*) for human γM-immunoglobulin. The molecular weights of the light chains are similar to those reported for light chains of the immunoglobulins in lemon shark (*11*) and mammals. Heavy chains on the dogfish immunoglobulins have molecular weights in the range reported (*9*) for μ-chains on mammalian immunoglobulins (approximately 70,000); this value is considerably higher than that reported (*12*) for γ-chains (approximately 55,000). The molecular weight of the α-chains from γA-immunoglobulin has not been reported and thus cannot be compared. A dogfish molecule composed of two light chains and two heavy chains would have a molecular weight of approximately 188,000. The observed value for the 7*S* immunoglobulin was 198,000 ± 6,000. Five units, each composed of two light and two heavy chains, would have a molecular weight of 940,000. The observed value for the 17*S* immunoglobulin was 982,000 ± 25,500. The amino compositions (Table 1) are consistent with the above assignment of chains within the dogfish immunoglobulin molecules.

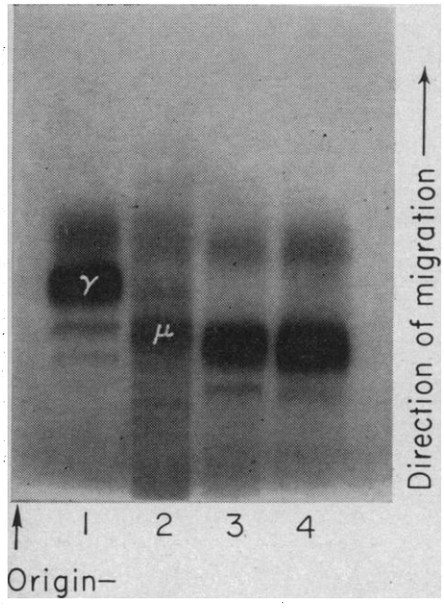

Fig. 1. Comparison of reduced alkylated immunoglobulins of dogfish and man by starch-gel electrophoresis in urea. Origin 1, human γG-immunoglobulin; origin 2, human γM-immunoglobulin; origin 3, dogfish 17*S* immunoglobulin; origin 4, dogfish 7*S* immunoglobulin. The letters γ and μ refer to the position of the heavy chains of human γG- and γM-immunoglobulins respectively. All samples were reduced and alkylated in the presence of 8*M* urea. Electrophoresis was performed in 8*M* urea-formate buffer according to the conditions of Edelman and Poulik (*3*).

Our data provide support for the conclusion that the 7*S* and 17*S* immunoglobulins of the smooth dogfish belong to the same class. This class resembles that of γM-immunoglobulins of the higher vertebrates. A report (*11*) on immunoglobulins in the lemon shark suggests that similar relations obtain in other elasmobranchs. As proposed (*1*), the μ-chains may have been the first of the heavy chains to have evolved. This is of interest in the light of a suggestion (*13*) that the structural genes for heavy chains may have arisen from duplication and mutation of genes coding for light chains.

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17. Supported by grants AM 04256 and AI 06985 from NIH and GB 3920 from NSF.

30 August 1966

Initial and Resultant Population Densities in Chickens between Brooding and Sexual Maturity

Abstract. *Seven flocks of chickens were raised in groups from weeks 5 to 9 after hatching. Initially the groups had the same number of chickens but they differed in population density. The number of birds that survived to week 9 was strongly related to the initial population density.*

Thiessen and Rodgers (*1*) summarized the results of a number of studies on factors that limit population density in infrahuman groups. Thiessen (*2*) subsequently proposed a correlational model to take into account the types of variables related to mortality, but as yet he has not been able to assign relative weightings to these variables. One of his factors, "prior experience with grouping," may facilitate survival under high population density. In this report we seek a precise relationship between initial and resultant densities. Since most of the theory and data seem related to mammalian groups, domestic fowl were used to supplement them.

Although Thiessen (*3*) prefers to make an assumption about the role of interspecific aggression, both field and laboratory studies (*4*) have demonstrated social interactive processes that result in a reduction in density. Most of these processes, however, apply to sexually mature or infantile-dependent members of infrahuman groups. Since little is known about density reduction among juveniles, we examined chicks after brooding and before sexual maturity. Between weeks 5 and 9 after in-