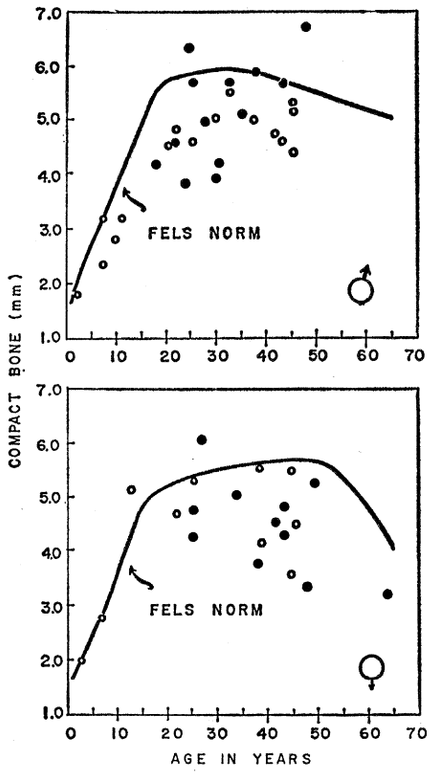

Fig. 1. Comparison of compact bone in American-born subjects of Chinese and Japanese ancestry (open dots), comparable subjects born abroad (black dots) and participants in the Fels Longitudinal Studies of Growth and Development (solid lines). Top, males; bottom, females. Both abscissas show age in years.

radiographs taken at a fixed (90 cm) tube-to-film distance. Replicability coefficients exceeded 0.98 (*2*).

As shown, the 51 Chinese and Japanese subjects had significantly less compact bone than the reference population at all ages ($\chi^2 = 24.0$). As a group they averaged 1.28 standard deviations below the Fels trend line. This tendency for diminished compact bone for subjects of Asiatic ancestry was true both for those born abroad (−1.37 S.D.) and those born in the United States (−1.19 S.D.). The nine children under 18 years of age similarly showed less compact bone at the middle-metacarpal site than the reference population.

In addition to the data on 51 subjects shown in Fig. 1, eight additional children of Chinese-Caucasian ancestry were studied. Though American-born, and from professional families of superior economic status, these children also fell below the Fels Institute averages for compact bone, though to a lesser degree (−0.66 S.D.) than the Chinese-Japanese group as a whole.

Compact bone formation is unquestionably impaired in children from Central America suffering from Kwashiorkor or in areas where native dietaries otherwise provide as little as 1.8 g of animal protein per day, as we have shown (*3*). Dietary deficiencies during the war years may account for the very low compact bone values here observed for seven of our Japanese subjects born between 1931 and 1946. However, the low levels of compact bone observed in both American-born and Asiatic-born Chinese and Japanese, their continuance in American-born children of Chinese families of superior economic status, and the intermediate levels of compact bone in children of Chinese-White ancestry obviously lead us to consider that the lesser compact bone of Asiatics is primarily a reflection of endogenous control mechanisms.

To this point we may cite parent-child and sibling comparisons of compact bone in more than 100 families in the Fels Longitudinal Studies, which indicate genetic mediation of compact bone in the second metacarpal, and further suggest mediation by the X chromosome (*2*).

Thus, while dietary studies of all subjects are currently in progress, and while occupation and handedness are being investigated further, we are inclined to attribute the lesser amounts of compact bone in Chinese and Japanese, here observed, more to genetic factors than to limiting nutrients.

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Molecular and Submolecular Localization of Two Isoantigens of Mouse Immunoglobulins

Abstract. *Three related classes of immunoglobulins (7S γ-, β_{2A}-, and γ_{1M}-globulin) in normal C3H and C57BL/6 mouse serums were isolated and examined for the isoantigens Iga-1 and Iga-2. The results indicate that the genetic locus determining the Iga-1 and Iga-2 antigens affects only a portion of the normal mouse immunoglobulin population (that is, 7S γ-globulin) and only the γ-specific part ("F" piece) of the 7S γ-globulin molecule.*

The immunoglobulins are a group of heterogeneous, structurally related serum proteins with which antibody activity has been associated. Recently, genetic polymorphism of mouse immunoglobulins has been recognized with the use of isoimmune antiserums (*1*). Two isoantigens, Iga-1 and Iga-2, under the control of allelic genes, have been described (*2*). The present study was undertaken to determine which class of immunoglobulin carries the antigens specified by the Iga-1 and Iga-2 locus and what molecular subunit has these isoantigenic characters.

Serums from normal and immune C3H/He mice (Iga-1) and C57BL/6 mice (Iga-2) were used for the preparation of 7*S* γ-globulins, β_{2A}-globulins, and 18*S* γ_1-macroglobulins. The 7*S* γ-globulins were isolated by block (zone) electrophoresis (*3*); 18*S* γ_1-macroglobulins and β_{2A}-globulins were obtained by a combination of block electrophoresis and filtration through Sephadex G-200 (*4*). The preparations of normal β_{2A}-globulins were contaminated with small amounts of 18*S* γ_1-macroglobulins. Myeloma β_{2A}-globulins, which are closely related to normal β_{2A}-globulins, were prepared by zone electrophoresis and diethylaminoethyl-cellulose chromatography of serums from C3H mice bearing the transplantable plasma cell tumors, 5647 and SPC-1 (*5*). The identity and purity of the fractions were evaluated by immunoelectrophoresis with rabbit antiserums prepared by immunization with mouse immunoglobulin fractions or normal mouse serum. Starch-gel electrophoresis and ultracentrifugation were also used to characterize representative fractions.

Purified 7*S* γ-, β_{2A}-, and 18*S* γ_1-macroglobulins from C3H mice were

compared by Ouchterlony double diffusion analysis (Fig. 1*A*). Polyvalent antiserums, prepared in rabbits immunized with globulin fractions of hyperimmune mouse serums, reacted to form a single precipitin band with each of the groups of mouse immunoglobulin. Each of the precipitin lines intersects with the adjacent component, indicating antigenic individuality of each immunoglobulin group. Separate tests (not shown) demonstrated that the three groups share common antigens detected by the rabbit antiserums.

The same C3H immunoglobulin preparations were tested for the presence of the Iga-1 antigen as shown in Fig. 1*B*. Iga-1 isoimmune serum was placed in the lower well and formed a precipitin band only with 7*S* γ-globulin, indicating that only the 7*S* γ-globulins of C3H mice have the Iga-1 antigenic determinants. The β_{2A} preparation shown is myeloma protein 5647; tests were also done with myeloma protein SPC-1 and normal β_{2A}-globulin (contaminated with small amounts of 18*S* γ_1-macroglobulin). These failed to react with the isoimmune serum, and thus provided additional evidence that β_{2A}-globulins lack the Iga-1 antigen.

Purified 7*S* γ-, β_{2A}-, and γ_{1M}-globulins were tested for the Iga-2 isoantigen (Fig. 1*C*). Isoimmune antiserum to Iga-2 (in the lower well) reacted only with 7*S* γ-globulin. This observation and those reported above for C3H immunoglobulins show that the Iga antigens are limited to the 7*S* γ-globulins.

Further studies of 7*S* γ-globulins were undertaken to determine the submolecular localization of the isoantigens. "Slow" S and "fast" F pieces of normal, 7*S* γ-globulins were prepared by treatment with papain and cysteine according to the method of Porter (*6*), except that the digestion times were 1 hour for C57BL proteins and 4 hours for C3H proteins. In Fig. 2 (lower half) the papain digest of C3H γ-globulin was examined by immunoelectrophoresis. Polyvalent rabbit antiserum to mouse γ-globulin, which was placed in the trough below the papain digest, formed two precipitin arcs, one with the more cathodal "slow" piece and another with the more anodal "fast" piece. Isoimmune antiserum to Iga-1 placed in the center trough of Fig. 2 reacts to form a precipitin line only with the "fast" piece. Similar results were obtained with immunoelectrophoretic tests of the papain digest of γ-globulin from C57BL mice, when isoimmune antiserum to Iga-2 was placed in the center trough.

Previous studies (with rabbit antiserums) have shown that antigenic determinants common to all the immunoglobulins are present on the "slow" piece and that determinants specific for the molecular class are present on the "fast" piece (*7*). Our data provide independent evidence for the class specificity of the "fast" piece.

Polymorphism of the immunoglobulins has been observed and studied in other species as summarized in Table 1. The Iga locus of mice is homologous to the Gm locus of man and is different from the 2 loci described in rabbits.

Discovery of additional isoantigenic markers on mouse immunoglobulins can be anticipated, and a combination

Table 1. The molecular and submolecular localization of determinants for the polymorphisms of the immunoglobulins of various species.

Locus	Molecular class			Papain digest subunit	
	7Sγ	γ_{1M}	β_{2A}*	Slow (I, II)	Fast (III)
		Rabbit (8)			
a	+	+		+	0
		Rabbit			
b	+	+		+	0
		Man (9)			
Inv	+	+	+	+	0
		Man			
Gm	+	0	0	0	+
		Mouse			
Iga	+	0	0	0	+

* Rabbit β_{2A}-globulin has not been examined for these determinants.

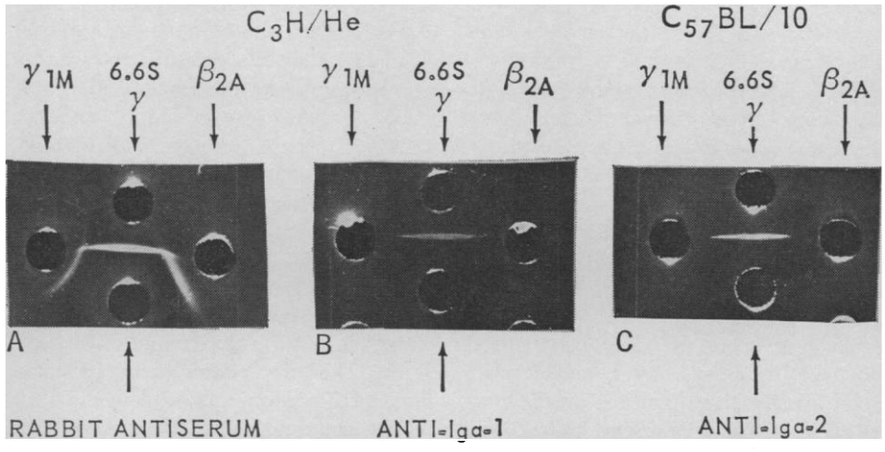

Fig. 1. Double diffusion tests of isolated mouse immunoglobulins. *A*, Strain C3H proteins tested with a polyvalent rabbit antiserum; *B*, C3H proteins tested with isoimmune antiserum to Iga-1; *C*, C57BL proteins tested with isoimmune antiserum to Iga-2. Isoimmune precipitin lines occur only between the 7*S*-globulins and the isoimmune serums.

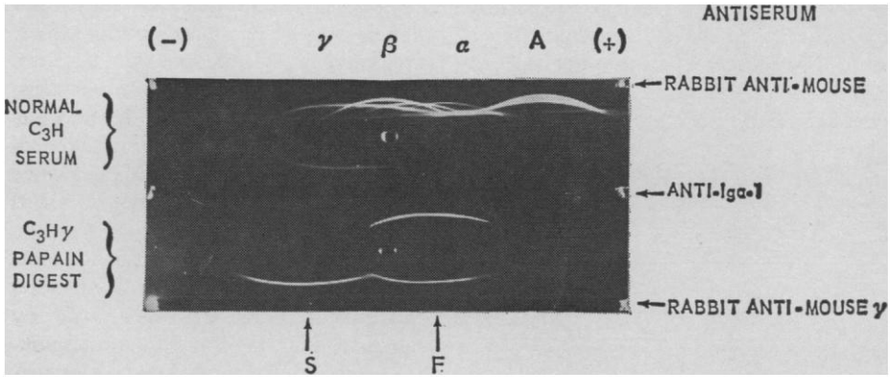

Fig. 2. Immunoelectrophoresis of normal C3H serum and papain treated C3H 7*S*γ-globulin. Isoimmune antiserum to Iga-1 and polyvalent rabbit antiserum to mouse globulin were added to the troughs as indicated. The isoimmune antiserum to Iga-1 reacted to form precipitin lines with the γ-globulin of normal serum and with the "fast" piece of papain treated 7*S* γ-globulin, indicating that the Iga-1 antigens are present on these components.

of genetic studies in inbred mice and submolecular characterization of the immunoglobulins should lead to further insight into the genetic-structural relationship of these important proteins.

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6 January 1964

Polymer Similar to Polydeoxyadenylate-Thymidylate in Various Tissues of a Marine Crab

Abstract. *Several species of a genus* Cancer *have a DNA that has a light buoyant density and that contains mainly deoxyadenylate and thymidylate. The presence of this polymer was demonstrated previously in testes and vas deferens. By a modified procedure for isolating DNA, the muscles, liver, and eggs of* Cancer borealis *are also shown to contain the deoxyadenylate-thymidylate-like polymer.*

Certain marine crabs both in the Atlantic Ocean (*Cancer borealis* and *Cancer irroratus*) (*1–3*) and in the Pacific Ocean (*Cancer antnnearius, Cancer gracilis,* and *Cancer magister*) (*4*) have a DNA component whose base composition is mostly deoxyadenylate-thymidylate. In the case of *C. borealis,* this polymer consists of 30 percent of the total DNA of the testes. The polymer was isolated free from the main DNA and was found to have a double helical structure and density and thermal hyperchromic shift similar to those of deoxyadenylate-thymidylate polymer which had been enzymatically synthesized (*2, 3*). The polymer acts as a primer for DNA polymerase, and the deoxyadenylate and deoxythymidylate residues are in alternate sequence (*5*). It contains 2.7 percent guanine and cytosine, and there is a tendency for deoxyguanylic residue to replace deoxyadenylic rather than thymidylic and for the deoxycytidylic residue to replace the thymidylic (*5*). The polymer also acts as an excellent primer for RNA polymerase isolated from *Micrococcus lysodeikticus* (*6*).

The existence of the extra DNA component in different tissues of the crab was not clear because of the difficulty of obtaining highly polymerized DNA from tissues other than testes and vas deferens. With a different method of DNA isolation, we have been able to characterize the DNA of the muscle, liver, and eggs of *C. borealis.* Centrifugation of the material in a CsCl gradient shows that the DNA from the muscle, liver, and eggs contains the polymer in approximately the same amount, and density identical to that of the light DNA component in the testes and vas deferens.

Live crabs were dissected, and the testes, claw muscle, liver, and eggs were washed separately with an EDTA-saline solution (0.1*M* ethylenediamine tetraacetate, 0.15*M* NaCl, *p*H 8.0). The testes were frozen immediately and kept in the freezer. The washed muscle, liver, and eggs were quickly frozen by dipping them into liquid nitrogen. The tissues were kept in liquid nitrogen until they were used for DNA isolation.

Table 1. Relative amounts of the main DNA and "dAT" in different tissues of *Cancer borealis.**

Tissues	Main DNA (%)	"dAT" (%)
Testes	69	31
Liver	68	32
Muscle (claws)	68	32
Eggs	66	34

* Calculated from tracings of ultraviolet absorption pictures of equilibrated DNA in the CsCl density gradient.

The procedure for isolating DNA from crab testes has been described (*2*). Approximately 20 g of muscle was ground in a chilled mortar with an occasional addition of liquid nitrogen. The frozen powder was mixed with 70 ml of cold EDTA-saline plus duponol (2.5 percent) in a teflon-pestle tissue grinder, while the temperature was kept below 4°C. Fourteen milliliters of 5*M* sodium perchlorate were added to the mixture. The suspension was shaken with a mixture of isoamyl alcohol and chloroform (4:96 by volume) at room temperature for 60 minutes. The mixture was centrifuged at 4°C at 5000*g* for 15 minutes. The top aqueous layer was shaken with a mixture of isoamyl alcohol, chloroform, and phenol (3:72:25) for 30 minutes and the mixture was centrifuged. The resulting top layer was then shaken with a mixture of isoamyl alcohol, chloroform, and phenol (2:48:50) and centrifuged. To the final aqueous portion, two volumes of 95 percent ethanol were added to precipitate nucleic acids. The precipitates were collected by centrifugation and dissolved in 10 ml of standard saline citrate (0.15*M* NaCl plus 0.015*M* sodium citrate) to which a preheated ribonuclease solution (*7*) was added, making the ribonuclease concentration 50 μg/ml. After incubating the mixture at 37°C for 30 minutes, one-fourth volume of 5*M* sodium perchlorate was added and shaken with an equal volume of a mixture of isoamyl alcohol, chloroform, and phenol (2:48:50) at room temperature for 15 minutes. Macromolecules were precipitated with ethanol as described, and the precipitates were dissolved in 5 ml of standard saline citrate. The solution had a total of 300 units of absorbance at 260 mμ. A portion of the solution (1 ml) was diluted with saline citrate to an absorbance at 260 mμ of 0.8 and applied to a one-layer methylated albumin-kieselguhr (MAK) column (*2*). The column was prepared with 10 ml of a suspension of MAK (*2*) and had a packed size of 1.5 cm in diameter and 1 cm in height. The charged