of product to precursor, shown in the last column of Table 2, is thus a measure of relative incorporation rate of ATP into liver RNA. Thus, at a dosage of 160 μg of actinomycin per 100 g of body weight, the change in incorporation rate was negligible. At doses that inhibit prothrombin synthesis (800 μg/100 g), the synthesis of RNA from ATP was inhibited about 80 percent.

These data suggest that vitamin K acts to induce prothrombin synthesis. Based on the model proposed by Jacob and Monod (*11*) the following hypothesis is advanced. In the absence of vitamin K a regulatory gene represses the activity of the operon concerned with the elaboration of the vitamin K-dependent clotting proteins, namely prothrombin, plasma thromboplastin antecedent, plasma thromboplastin component, and proconvertin. Vitamin K may then act to derepress the operator by combining with the repressor molecule. The structural gene components of the operon are thus freed to elaborate their respective messenger RNA's which result in the synthesis of their respective proteins. The effect of actinomycin D in blocking the action of vitamin K in these experiments is consistent with the known action of actinomycin in blocking DNA-dependent RNA synthesis (*5*). This hypothesis also explains the failure of vitamin K to act in hereditary hypothrombinemic states (*12*) in which it may be assumed that the structural gene components of the operon are defective.

This work suggests the possibility that all of the fat-soluble vitamins operate to control the synthesis of specific proteins and enzymes in the highly differentiated mammal. This speculation is strengthened by the parallel similarity of the other fat-soluble vitamin deficiency diseases to genetically conditioned disorders. Evidence already exists that some of the chemically related steroid hormones act at the genetic level (*13*).

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Dependence of a Gm(b) Antigen on the Quaternary Structure of Human Gamma Globulin

Abstract. *The antigens associated with the Gm(b) factor of human 7S γ-globulin differ in Caucasoids and Negroids. The studies reported here show that an antigen, Gm(b^w), associated with the Gm(b) of whites but not with that of Negroes, is present on the S (slow) fragment of 7S γ-globulin obtained by digestion with papain or pepsin. All other Gm antigens thus far studied have been found on the F (fast) fragment. The antigens Inv(a) and Inv(b) were detected on isolated L-chains, and Gm(a) and Gm(b) were detected on isolated H-chains, but Gm(b^w) was detected on neither. Recombining the H- and L-chains restored activity for Gm(b^w) comparable to that of intact γ-globulin.*

The hereditary antigens of human 7*S* γ-globulin are determined by codominant alleles at two different loci, Gm and Inv (*1*). Studies on the distribution of Gm and Inv antigens on fragments of 7*S* γ-globulin produced by papain digestion have shown that the Gm(a) and Gm(b) antigens are located on the F (fast) fragment, while the Inv(a) and Inv(b) antigens are located on the S (slow) fragment (see *2*).

Steinberg and Wilson (*3*) have shown that Gm(b+) Caucasians possess an antigen, termed Gm(b^w), which is absent in Gm(b+) Negroes. This paper is a report of our first studies of the distribution of the Gm(b^w) antigen on enzymatically produced fragments of human 7*S* γ-globulin.

Gamma-globulin (7*S*) was isolated from the serums of four Gm(b^w+) donors by elution from diethylaminoethyl (DEAE) cellulose according to the method of Sober and Peterson (*4*). The γ-globulin was digested with papain in the presence of cysteine according to the method of Porter (*5*). The F and S fragments obtained were separated by chromatography on DEAE-cellulose. The S fragment preparations were further purified by gel filtration with Sephadex G-100. Immunoelectrophoresis and double diffusion in agar (Ouchterlony technique) failed to reveal contaminating material in the F fragment preparations. Some contaminating, undigested 7*S* γ-globulin was detected in the S fragment preparations by the Ouchterlony technique, but not by immunoelectrophoresis.

As already noted, the Gm(a) and Gm(b) antigens are located on the F fragment, while the Inv(a) and Inv(b) antigens are on the S fragment (*2*). We have confirmed these observations for the antigens detected by the standard reagents, but we have consistently found the Gm(b^w) antigen on the S fragment. This is illustrated in Table 1 by data obtained from one of the donors.

Similar results have been obtained when 7*S* γ-globulin was digested with pepsin according to the method of Nisonoff *et al.* (*6*). This method produces an S fragment similar to that produced by papain digestion, but no F fragment is recovered (*6, 7*). Samples of purified pooled 7*S* γ-globulin and 7*S* γ-globulin isolated from two Gm(b^w+) donors were digested with pepsin for 18 to 48 hours. Traces of undigested γ-globulin were detected by the Ouchterlony technique in samples digested for 18 hours, but not in samples digested for 48 hours. After 48 hours of digestion of 7*S* γ-globulin from a Gm(a—b+b^w+), Inv(a—b+) donor, Gm(b) was not detectable at a protein concentration of 8.8 mg/ml. On the other hand, Gm(b^w) was detected at a protein concentration of 0.05 mg/ml, as compared to 0.10 mg/ml for intact 7*S* γ-globulin, and Inv(b) was detected at 0.03 mg/ml as compared to 0.009 mg/ml for intact γ-globulin (*8*) (Table 2).

Table 1. The effect of papain digestion of 7*S* γ-globulin on the Gm and Inv antigens of a Gm(a+b+b^w+), Inv(a+b+) donor.

Factor	Lowest protein concn. for antigen detection (mg/ml)		
	Intact 7*S*γ	F fragment	S fragment
Gm(a)	0.009	0.010	2.8*
Gm(b)	0.105	2.7	N.D.† at 2.7
Gm(b^w)	0.084	N.D. at 2.8	0.175
Inv(a)	0.105	N.D. at 2.7	0.700

* Probably due to contamination with undigested γ-globulin. † N.D., not detected.

Similar results were obtained when 7*S* γ-globulin from a Gm(a+b+b^w+), Inv(a+b+) donor was digested with pepsin for 48 hours (Table 2). The Gm(a) and Gm(b) factors were not detected at protein concentrations of 15 mg/ml while Gm(b^w) and Inv(a) were detected at 0.050 mg/ml and 0.375 mg/ml, respectively. These data also indicate that the Gm(b^w), Inv(a), and Inv(b) antigens are located on the S fragment.

According to the model of 7*S* γ-globulin proposed by Porter (*9*), an intact molecule of γ-globulin consists of four polypeptide chains, two heavy or H-chains, each with a molecular weight of approximately 50,000, and two light or L-chains, each with a molecular weight of approximately 20,000 (*10*). The F fragment produced by papain digestion appears to be composed solely of a portion of the H-chains, while the S fragment consists of a portion of the H-chains as well as the whole of the L-chains. Therefore, there are three possibilities for the location of the Gm(b^w) antigen. The antigen may be located on the L-chain, located on the portion of the H-chain within the S fragment, or be the result of an interaction of the H- and L-chains within the S fragment.

Table 2. The effect of pepsin digestion of 7*S* γ-globulin on the Gm and Inv antigens.

Factor	Lowest protein concn. for antigen detection (mg/ml)	
	Before digestion	After digestion
A. Donor's type: Gm(a−b+b^w+), Inv(a−b+)		
Gm(b)	0.105	N.D.* at 8.8
Gm(b^w)	0.100	0.050
Inv(b)	0.009	0.030
B. Donor's type: Gm(a+b+b^w+), Inv(a+b+)		
Gm(a)	0.009	N.D. at 15
Gm(b)	0.105	N.D. at 15
Gm(b^w)	0.100	0.050
Inv(a)	0.105	0.375

* N.D., not detected.

The H- and L-chains were obtained by reduction and alkylation of 7*S* γ-globulin from a Gm(a + b + b^w +), Inv(a + b +) donor according to the method of Fleischman, Pain, and Porter (*11*). Analysis of these preparations by Ouchterlony technique with specific antiserums, against Type I and Type II Bence Jones proteins and against the F fragment, revealed no contaminating material (*12*). The Inv(a) and Inv(b) antigens were detected in the L-chain preparation at a protein concentration comparable to that of intact 7*S* γ-globulin—0.056 mg/ml for the detection of Inv(a) in the L-chain preparation compared to 0.028 mg/ml for its detection in 7*S* γ-globulin, and 0.007 mg/ml compared to 0.009 mg/ml, respectively, for Inv(b). The Gm(a), Gm(b), and Gm(b^w) antigens were not detected in the L-chain preparation at a protein concentration of 1.4 mg/ml.

The Gm(a) and Gm(b) antigenicity of the H-chain preparation compared favorably to that of intact 7*S* γ-globulin, namely 0.007 mg/ml were required for the detection of Gm(a) in the H-chain preparation compared to 0.009 mg/ml for intact 7*S* γ-globulin and 0.421 mg/ml compared to 0.105 mg/ml, respectively, for Gm(b). Inv(a) was not detectable in the H-chain preparation at a concentration of 1.8 mg/ml; Inv(b) was not tested for. The Gm(b^w) antigen, detected at 0.026 mg/ml in intact 7*S* γ-globulin, was not detectable in the H-chain preparation tested at 1.8 mg/ml.

The antigen Gm(b^w) was not detected in β_{2M}-globulin isolated from the serum of a Gm(a+b+b^w+) donor. The L-chains of 7*S* γ-, β_{2A}-, and β_{2M}-globulin appear to be very similar electrophoretically and antigenically, while the H-chains show marked differences from one another (*13*). Hence the absence of Gm(b^w) from β_{2M}-globulin confirms our findings on the isolated L-chains.

The absence of the Gm(b^w) antigen from both isolated H- and isolated L-chains indicated that neither chain alone was responsible for the Gm(b^w) antigenicity of the molecule. If the Gm(b^w) antigen were the result of an interaction between the H- and L-chains, and if the interchain disulfide bonds were not essential for the expression of the antigen, reconstituted molecules produced by mixing isolated H- and L-chains from a Gm(b^w+) donor might result in a return of Gm(b^w) antigenicity. Isolated H- and L-chains were recombined according to the method of Edelman *et al.* (*14*). The Gm(b^w) antigen was detected in the recombined H- and L-chains at protein concentrations similar to those at which the antigen was detected in intact 7*S* γ-globulin, 0.050 mg/ml compared to 0.100 mg/ml, respectively. Gm(b^w) was not detected in preparations of isolated H- and isolated L-chains subjected to the same conditions under which mixtures of H- and L-chains regained their Gm(b^w) antigenicity. It appears, therefore, that this genetically determined antigen is an expression of the quaternary structure of the 7*S* γ-globulin molecules (*15*).

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