TABLE II. The Concentration of Ca in Plasma Water, Ultrafilterable Plasma, and Cisternal Cerebrospinal Fluid in the Rat.[a]

Wt. range of rats (gm)	Plasma sp gr (mean)	(A) Plasma Ca (meq/kg of H_2O)	(B) Plasma ultra-filterable Ca (meq/liter)	B/A × 100 (%)	CSF Ca (meq/kg)
120–130	1.023	5.64 ± 0.05 (10)	3.45 ± 0.25 (8)	61.8 ± 4.77 (8)	2.89 ± 0.14 (10)
320–380	1.025	5.65 ± 0.05 (8)	3.43 ± 0.06 (7)	60.8 ± 0.39 (7)	2.77 ± 0.04 (6)

[a] Values expressed as mean ± SE; (no. of animals given in parentheses).

effect on any of the results. The CSF-Mg exceeded UF-Mg, and CSF-Ca was less than UF-Ca, indicating the existence of a blood–CSF concentration gradient for these cations in the rat.

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Study by Peptide Mapping of Antihapten Antibody of Varying Affinity from Individual Rabbits* (33065)

BARBARA LISOWSKA-BERNSTEIN, GREGORY W. SISKIND,[1] AND MICHAEL E. LAMM[1]
Departments of Pathology and Medicine, New York University School of Medicine, New York, New York

It is well known that antihapten antibody is heterogeneous with regard to its affinity for the homologus haptenic determinant (1–4). It is further known that the average affinity of the antihapten antibody present in the serum of an immunized animal increases progessive-

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ly with time after immunization (4). It has been reported by McGuigan *et al.* (5) that the antihapten antibodies formed early and late in the immune response of an individual rabbit differ in amino acid composition.

The available data is consistent with the concept that antihapten antibody produced by an individual animal consists of a highly heterogeneous, continuously varying population of molecules. However, it is also possible that the observed variations in affinity are a reflection of a small number of distinct, relatively homogeneous subpopulations of antibody molecules, each with a different affinity. Thus, the change in average affinity with time after immunization might represent a change in the relative proportions of a small number of different homogeneous antibody subpopulations. If heterogeneity were a reflection of the presence of a small number of distinct homogeneous antibodies, one might expect to find differences upon comparing peptide maps of high and low affinity antibody preparations. On the other hand, if antibody of a given specificity is a highly heterogeneous population of molecules, peptide maps, which reflect only constant portions of the primary structure of a protein, would not be expected to distinguish between high and low affinity antibody preparations.

In the present report we have examined peptide maps of $F(ab')_2$ fragments of high and low affinity antihapten antibody obtained from individual rabbits. No differences were detected, suggesting a high degree of heterogeneity rather than a small number of discrete antibody subpopulations.

Materials and Methods. Antigens and haptens. Dinitrophenylated bovine gamma globulin ($DNP_{50}BGG$) and dinitrophenylated bovine fibrinogen ($DNP_{150}BF$) were prepared by the reaction of dinitrofluorobenzene with protein under alkaline conditions essentially according to the method of Eisen (6). The concentration of protein was determined by drying a known volume to constant weight at 100°C.

Preparation of antibody. Rabbits homozygous at both the a and b loci (1,1:4,4) were obtained from Jackson Laboratories, Bar Harbor, Maine, and immunized by subcutaneous injection of 5 mg of DNP-BGG emulsified in complete Freund's adjuvant into the 4 footpads and the back of the neck. Animals were bled 2 and 6 weeks after immunization. Antibody concentration was measured by the quantitative precipitin reaction using DNP-BF as antigen according to methods of Farah *et al.* (7). Antibody was purified by 2,4-dinitrophenol extraction of specific precipitates of antibody with DNP-BF as described by Farah *et al.* (7). Hapten was removed by dialysis and chromatography on Dowex 1-X8. Antibody from individual animals was separated into fractions differing in average affinity by the serial precipitation method of Eisen and Siskind (4), and antibody affinity for DNP-lysine was determined by the fluorescence quenching technique of Velick *et al.* (8). The procedure employed was identical to that described in detail by Eisen and Siskind (4).

Peptide mapping. The $F(ab')_2$ fragments, obtained by pepsin digestion of purified anti-DNP antibody at pH 4.5 for 15 hours at 37°C as described by Nisonoff (9) and lacking Fc antigenic determinants by agar gel diffusion with sheep antirabbit γ globulin, were extensively reduced with 0.3 *M* β-mercaptoethanol in 7 *M* guanidine·HCl at pH 8.2 and alkylated with iodoacetamide. Tryptic digests were mapped by the method of Katz *et al.* (10) as described previously (11). The $F(ab')_2$ fragments, rather than whole antibody molecules, were used to avoid the large number of peptides contributed by the Fc fragments which would be independent of variations in affinity.

Results. Antibody produced by the rabbits at 2 and 6 weeks after immunization was studied. Each sample was separated by serial precipitation into 2 or 3 fractions differing progressively in affinity. That adequate fractionation according to affinity was obtained was confirmed by measurement of the affinity of each sample for N-ϵ-2,4-dinitrophenyl-L-lysine (DNP-lysine). The results are presented in Table I. It is clear that, as reported previously (4), antibody affinity increased with time after immunization and that sequential precipitation yielded a series of fractions differing markedly in affinity.

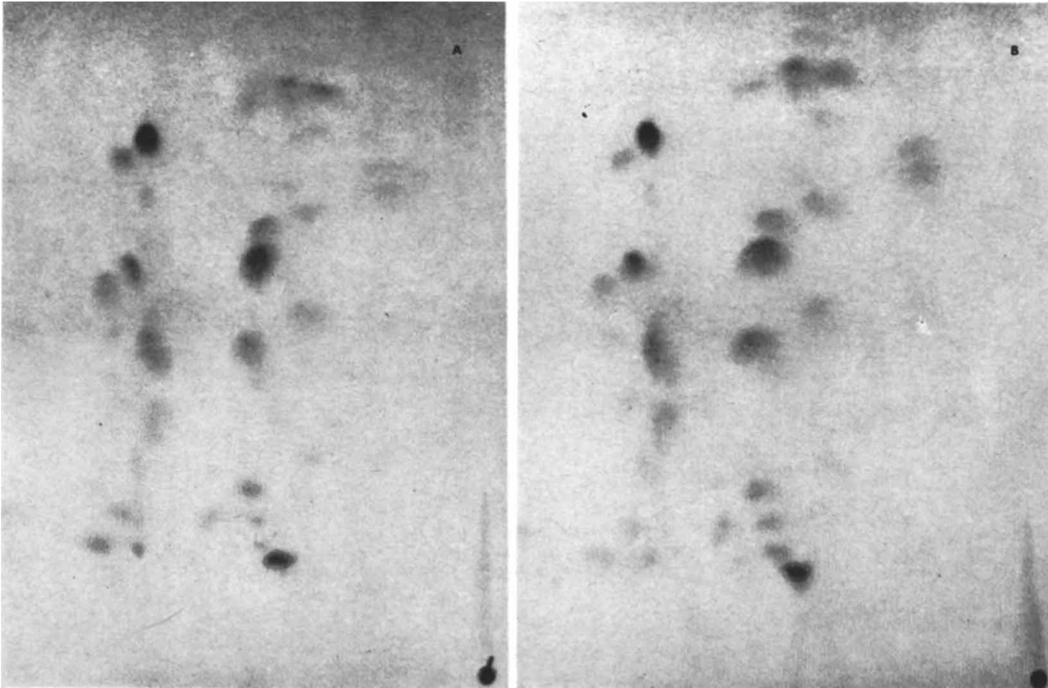

FIG. 1. Peptide maps of low (A) and high (B) affinity $F(ab')_2$ fragments obtained from the same rabbit. The origin is at the lower right. Chromatography is from right to left, and electrophoresis is from bottom to top.

Peptide maps were made with the $F(ab')_2$ fragments of the antibody preparations described in Table I. All maps were identical despite the fact that some of the antibody fractions differed markedly in affinity. Peptide maps of fraction 2 from the early bleeding of rabbit 3 and of fraction 1 from the late bleeding of the same animal are illustrated in Fig. I. Despite the extreme difference in affinity (5 orders of magnitude) of these two antibody preparations, no difference in peptide maps are observed.

Discussion. The $F(ab')_2$ fragments obtained from antibody fractions of markedly differing affinity produced by individual rabbits towards the DNP group were found to yield identical peptide maps. It has been previously reported (5) that anti-DNP antibodies formed early and late in immunization differ in amino acid composition. From the peptide mapping results it can be tentatively concluded that these differences lie in a highly variable portion(s) of the $F(ab')_2$ fragment. The failure of $F(ab')_2$ fragments of high and low affinity antibody fractions to yield distinctive peptide maps suggests that the heterogeneity of and variations in affinity

TABLE I. Affinity of Antibody Fractions for DNP-Lysine.

		K_0 (liters/mol)[b]	
Rabbit no.	Fraction[a]	2-week bleed	6-week bleed
1	1	8.5×10^6	1.7×10^8
	2	—	5.6×10^6
2	1	1.8×10^7	7.6×10^{10}
	2	1.6×10^6	1.9×10^9
	3	—	1.3×10^8
3	1	3.0×10^7	10^{11}
	2	3.0×10^6	—
	3	—	1.9×10^8

[a] Antibody fractions obtained by serial precipitation by addition of small amounts of antigen (see "Methods").

[b] Association constants measured by fluorescence quenching at 20°C in 0.15 M NaCl, 0.01 M K phosphate buffer, pH 7.3.

which are observed cannot be explained on the basis of the existence, in varying proportions, of a limited number of homogenous antibody subpopulations. Rather, purified antihapten antibody apparently consists of a highly heterogenous population of molecules. Similar conclusions have been reached by several previous workers (12,13).

Summary. Peptide maps of $F(ab')_2$ fragments of anti-DNP antibody fractions differing markedly in affinity for DNP-lysine were indistinguishable, suggesting that the structural differences corresponding to the differences in affinity were located in a highly variable portion of the antibody molecule.

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Hepatotoxicity of Phenothiazines *in Vitro* as Measured by Loss of Aminotransferases to Surrounding Media* (33066)

CARLOS A. DUJOVNE, ROBERT LEVY, AND HYMAN J. ZIMMERMAN
Liver and Metabolic Research Laboratory, Veterans Administration Hospital and the Department of Medicine, The George Washington University School of Medicine, Washington, D.C.

The administration of chlorpromazine (CPZ), 10-(dimethylaminopropyl)-2-(chlorophenothiazine hydrochloride) leads to jaundice in 1–5% of the recipients (1). This phenomenon has been considered a manifestation of individual host idiosyncrasy or hypersensitivity to the drug. A closely related compound, promazine (PZ), 10-(dimethylaminopropyl)-(phenothiazine hydrochloride) however, has been attended by a very rare occurrence of jaundice, although extrahepatic manifestations of hypersensitivity occurs with both of these phenothiazines (1). Accordingly, the likelihood has been considered that the adverse effect CPZ may have upon the liver is aggravated by the coincidence of hypersensitivity. Some support for this view is provided by the demonstration that almost 50% of patients who receive CPZ develop some hepatic dysfunction (2–4). Previous studies utilizing a known hepatotoxin (carbon tetrachloride) demonstrated cytotoxicity in an *in vitro* system (5). The present study using an *in vitro* assay was undertaken to compare the relative adverse effects of CPZ and PZ. Leakage of intracellular enzymes which was presumed to result from injury to cellular membranes was utilized as an index of heptotoxicity.

Materials and Methods. Albino New Zealand strain rabbits were sacrificed by cervical fracture, their livers were removed and rinsed in cold saline. The tissue was blotted dry and from small chunks of tissue, slices were prepared using the Stadie-Riggs microtome. An intact tissue slice, weighing 50 mg

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