Poliovirus Type 1: Neutralization by Papain-Digested Antibodies

Abstract. *Papain-digested rabbit antibody (Porter's fractions I and II) can neutralize poliovirus. Neutralizing capacity after digestion ranged from 35 to 45 percent of that of the undigested antibody. No definite dissociation of the antibody fragments from the virus was observed after the reaction mixture had been diluted in a neutral medium.*

Papain-digestion of 7*S* rabbit antibodies yields three fragments that can be separated by chromatography on carboxymethylcellulose (*1*). Porter fragments I and II, although not capable of precipitating homologous antigen, show antibody activity; they combine with antigen and inhibit precipitation by undigested antibody. Porter fragment III lacks any immunological activity toward the specific antigen and seems to be responsible for certain other biological properties of the antibody molecule, such as complement-fixation (*2*) and "skin-fixation" in passive cutaneous anaphylaxis (PCA) and reverse passive cutaneous anaphylaxis (RPCA) (*3*).

Monovalent fragments I and II obtained from purified rabbit antihapten antibody failed to sensitize the skin of guinea pigs for PCA and RPCA, whereas Porter fragment III produced RPCA reactions (*3*). According to Ovary and Karush this failure may have been due to the inability of fragments I and II to affix to the guinea pig tissue (monovalent fragments diffuse from the injection site faster than the intact antibody); the fixation site of the antibody molecule seems to be present on Porter fragment III.

Baxter and Small (*4*) reported that papain-digested antibodies to rat kidney did not cause nephritis, suggesting that this failure may have been due to either the inability to fix complement or the result of the low molecular weight of the fragments.

Three basic differences must be considered in comparing the biological qualities of antibody fragments I and II with intact 7*S* antibody. Fragments I and II have a molecular weight of about 50,000; they are monovalent, possessing only one combining site and they do not fix complement. The 7*S* rabbit antibodies have a molecular weight of 160,000; they are divalent and are able to fix complement.

With regard to the capacity of 3.5*S* antibody fragments to neutralize virus infectivity, Lafferty (*5*) found that they could neutralize influenza A virus even though the fragments, unlike the intact antibody, dissociate completely from the virus on dilution of the reaction mixture in neutral saline medium. On the other hand, papain-digestion of rabbit antibodies against type 5 adenovirus resulted in loss of neutralizing capacity (*6*); the fragments, however, were still able to inhibit hemagglutination by this type of virus.

Rabbit antiserum against poliovirus type 1 was prepared by repeated intravenous and intramuscular injection of virus grown in cultured monkey kidney cells; serums from several animals were pooled. The γ-globulin fraction of the serum was isolated by five consecutive precipitations with 1.4*M* ammonium sulfate. The fraction was dissolved in buffered saline (*p*H 7.2), treated with 0.1*M* mercaptoethanol for 4 hours in order to destroy 19*S* antibodies, and dialyzed against a buffered saline until free of mercaptoethanol.

Digestion with papain (*7*) was performed as described by Porter. Fragment III was allowed to crystallize in the cold before removal by centrifugation. The supernatant contained fragments I and II that showed a sedimentation coefficient of about 3.5*S*. In immunoelectrophoresis of this fraction with rat antiserum to rabbit 3.5*S* fragments, two precipitation lines appeared, whereas immunoelectrophoresis of intact rabbit γ-globulin and the rat antiserum showed only one line (Fig. 1).

Titrations of infectivity ($TCID_{50}$) were carried out with rhesus monkey kidney cultured in tubes in the usual manner (*8*); the titers were calculated by the method of Reed and Muench (*9*). Neutralization tests were performed by mixing portions of serial twofold dilutions of antiserum with poliovirus type 1, strain Mahoney, containing about 200 $TCID_{50}$ per 0.5 ml; the reaction mixture was incubated for 4 hours at 37°C and overnight at 4°C. Each of a series of five to six tubes per serum dilution was inoculated with 1 ml of this mixture. Further dilution of the antibody-virus mixture after incubation was avoided. In order to study the kinetics of virus inactivation by undigested and papain-digested antibodies, mixtures of equal concentrations of a virus suspension ($10^{5.5}$ $TCID_{50}$ per 1.0 ml) and digested and undigested antibodies were prepared, and incubated at 37°C. At various time intervals, samples were removed from the reaction mixture, immediately diluted in chilled 0.5 percent solution of lactalbuminhydrolyzate (*10*) in Hanks balanced salt solution, and titrated for residual infectivity.

The fraction containing 3.5*S* fragments I and II neutralized poliovirus infectivity, but this neutralizing capacity was reduced to 35 to 45 percent by papain-digestion. Kinetic studies of

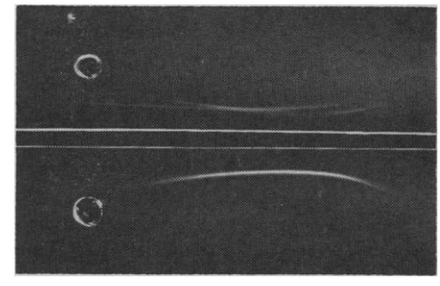

Fig. 1. Immunoelectrophoresis of 3.5*S* papain-digested rabbit γ-globulin fractions I and II (upper) and of undigested rabbit γ-globulin (lower). Antiserum against rabbit 3.5*S* fragments, fractions I and II combined, was obtained from the rat.

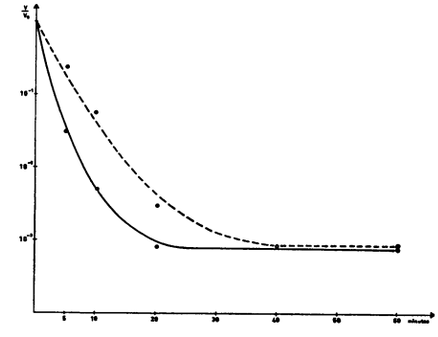

Fig. 2. Rates of neutralization of poliovirus type 1 by 3.5*S* fragments (fractions I and II combined) and by undigested antibody. At indicated time intervals, samples were removed from the reaction mixture ($10^{5.5}$ $TCID_{50}$ per milliliter, plus intact or papain-digested antibody of equal neutralizing capacity) and titrated for residual infectivity. V, virus concentration at indicated time; V_0, virus concentration at time zero; solid line, undigested rabbit antibody against poliovirus type 1; broken line, papain-digested antibody, fractions I and II.

virus inactivation by the digested and undigested antibodies were performed according to the procedure of Lafferty (*5*).

As shown in Fig. 2, no definite dissociation of antibody fragments from the virus was observed when the reaction mixture was diluted in a neutral medium. Furthermore, under the same conditions there was no evidence of dissociation when combined ^{32}P-labeled virus and monovalent antibody fragments were subjected to chromatography (*11*).

Thus, the neutralizing capacities of papain-digested antibodies against three different types of virus appear to differ. Type 5 adenovirus could not be neutralized by univalent (adenovirus) 3.5*S* antibody fragments. Fragments derived from rabbit antibody against influenza A virus showed neutralizing ability with influenza A virus, but the complex dissociated almost completely on dilution in a neutral saline medium. Our experiments demonstrate the neutralizing capacity and the irreversible binding of the antibody fragments to poliovirus particles.

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Bee Venom Tolerance in White Mice in Relation to Diet

Abstract. *In white mice the consumption of a high protein diet either just before, or over a period of 3 days prior to, the injection of venom from the honey bee* Apis mellifera *L. markedly increases the number of deaths. Conversely, a period of starvation or a nonprotein diet fed to white mice for 3 days prior to the injection of bee venom significantly reduces the number of deaths.*

A review of the literature indicated that no studies have been made on the relation of the composition of diet and time of feeding to the tolerance or detoxification of insect poisons in mammals, or both. Our experiments were undertaken to establish these relations.

The venom used in our experiments was collected in July 1963 by the method of Benton, Morse, and Stewart (*1*). The venom was a composite sample collected over a period of 1 week from 25 hives of bees. A stock solution was prepared by dissolving 200 mg of the pooled venom as completely as possible in 50 ml of 0.85 percent saline solution. The solution was filtered, sterilized in an autoclave, and frozen at −15°C in 10-ml serological vials. The number of micrograms of solid per milliliter was determined by drying and weighing a known volume of the venom. The stock solution contained 5 μg of venom per milliliter.

Female white mice (23 to 28 g) of the Swiss Webster strain were kept in an environmental growth chamber in 12 hours of light and 12 hours of darkness at 23.5° ± 0.5°C and a relative humidity of 65 percent ±1.5 percent. At 8:00 A.M. and 5:00 P.M. they were offered 2.5 g of rodent food per mouse. When special diets were substituted for the rodent food, the mice were offered an equivalent amount of food on the same schedule. The diets, obtained from Nutritional Biochemicals Corporation (*3*), were placed in contamination-free feeders to which the mice had access. Distilled water was available to the mice at all times.

The mice (except where otherwise stated) were injected by the intraperitoneal route as described by Campbell *et al.* (*2*). Six-tenths centimeter, 27-gauge needles with 0.25-ml syringes were used. All mice were weighed to the nearest 0.1 g prior to injection (10 to 20 minutes). The volume of bee venom administered was adjusted for each mouse on the basis of this body weight.

Fifteen mice were used for each determination, and each experiment was replicated three times. In experiments where the mice were injected immediately after feeding, 0.3 g was deducted from the weight of each mouse to compensate for the food which it had eaten, since this was found to be the maximum amount of food consumed by a series of mice feeding in a 25- to 30-minute period. All mice were injected with 0.21 μg of bee venom per gram of body weight. This is the concentration required to kill 5 mice out of a total of 15 mice in a 24-hour period under normal conditions (normal diet and no starvation period prior to feeding). All mice were injected at 3:00 P.M. to compensate for possible fluctuations due to daily rhythm.

Table 1. The tolerance of white mice to bee venom related to the time of feeding and diet. A, Starvation 18.5 hours prior to feeding; B, food available at all times; C, starvation 6 hours prior to injection. Three replications in each schedule.

Diet	Feeding schedule	Number dead after 24 hours (three replications)		
Peanut butter	A	11	10	11
Normal (protein, 27%)	B	5	5	5
Normal (protein, 27%)	C	1	0	2

Table 2. The relation of diet to the tolerance of white mice to bee venom. Fifteen mice were used in each experiment. The animals in group A were fed 20 minutes prior to injection after an 18-hour period of starvation, and those in group B were fed for 3 days prior to injection. There were three replications in each group. The number of deaths are recorded in the table.

Group A (No.)			Group B (No.)		
		High protein, 64%			
9	10	10	11	12	10
		High fat			
6	5	5			
		High carbohydrate			
5	4	5			
		No protein			
4	5	5	2	2	0
		Normal protein, 27%			
			5	5	4