Table III. Urea, dioxane, glycol, polyethylene sulfonate (PES), 2,4-dinitrophenol (DNP), and trypsin inhibited superprecipitation to some extent. The individual effect of these agents on dystrophic MyB suspensions was similar to that of normal controls.

Barany *et al.* (8) found no changes in the amino acid composition, aderosine triphosphatase activity and actin binding ability of MyA obtained from dystrophic chicken and mouse. Our results indicate no alteration in the "test tube contraction" of dystrophic MyB suspensions obtained from three different sources.

Summary. The sensitivity of myosins obtained from dystrophic rats and chickens to trypsin and pronase digestion were identical. The effect of urea and ethylene glycol on this proteolysis was the same with normal and dystrophic myosins. The superprecipitation of MyB suspensions obtained from the same dystrophic and normal animals was also similar in the absence and presence of protein denaturing agents, interaction inhibitors, and trypsin.

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Inhibition of Arylamidase by Rabbit Antiarylamidase Antibody* (32818)

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It has been shown that specific antibodies to enzymes will inhibit enzymatic activity (1). This report concerns the effect of rabbit antibody on an arylamidase prepared from cell-free extracts of *Neisseria catarrhalis*. Arylamidases are widely distributed in nature; they hydrolyze amino acyl-β-naphthylamines to yield the constituent amino acid and β-naphthylamine (βNA). The antibodies to this enzyme were detected by gel diffusion and quantitative precipitin tests. The antibody effectively inhibited arylamidase when enzyme was preincubated with antibody prior to addition of substrate as well as when all three were combined simultaneously. This study analyzes the nature of the inhibition caused by the rabbit antiarylamidase.

Materials and Methods. The growth of *N. catarrhalis* (ATCC 8176) and preparation of cell-free extracts were as previously reported by Folds and Behal (2). Arylamidase activity was assayed by determining liberated βNA with a Bratton-Marshall type procedure described by Behal *et al.* (3). Purification of arylamidase was accomplished by salt fractionation, DEAE cellulose and calcium phosphate chromatography according to Behal and Folds (4).

Antisera were prepared by intramuscular injection of purified *N. catarrhalis* arylamidase, which had been incorporated into

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Freund's adjuvant. Female New Zealand white rabbits each received three injections of 50–100 μg of protein on every third day. Ten days after the last injection the rabbits were bled, the blood was allowed to clot, and the serum collected.

Ouchterlony gel diffusion techniques as described by Campbell (5) were utilized. Rabbit γ-globulin was prepared from whole serum by chromatography on DEAE cellulose as described by Sober (6). The γ-globulin in the eluted fractions was detected in gel diffusion using a specific sheep antirabbit globulin (Cappel Laboratories).

Results and Discussion. Rabbit antisera were obtained which gave single precipitin lines on Ouchterlony gel diffusion plates when reacted with purified preparations of *N. catarrhalis* arylamidase (purified 548-fold). Because of the presence of an arylamidase in rabbit serum it was advantageous to purify rabbit globulin to demonstrate the effect of antibody on the bacterial enzyme. Arylamidase free rabbit γ-globulin was separated from the whole animal serum by DEAE cellulose chromatography.

An arylamidase preparation was precipitated by specific antibody in a quantitative precipitin reaction as described by Campbell (5). Serial dilutions of purified arylamidase were incubated with constant amounts of purified rabbit antiarylamidase γ-globulin; the γ-globulin concentration in each mixture was 1.0 mg/ml and the highest arylamidase concentration was 100 μg/ml. After standing 30 min at 37°C the incubation mixtures were maintained at 4°C for 42 hours. The mixtures were then centrifuged and the supernatants were collected and assayed for arylamidase activity. They were compared with controls in which normal rabbit γ-globulin replaced the immune globulin in the incubation mixture. The results were shown in Fig. 1. The antibody inhibited arylamidase while normal rabbit globulin did not.

The effect of antibody on enzymatic hydrolysis of L-alanine βNA was measured by two methods. Purified rabbit globulin was preincubated for 30 min with a purified preparation of arylamidase, after which substrate was added and the rate of hydrolysis was determined. In the second, antibody, enzyme,

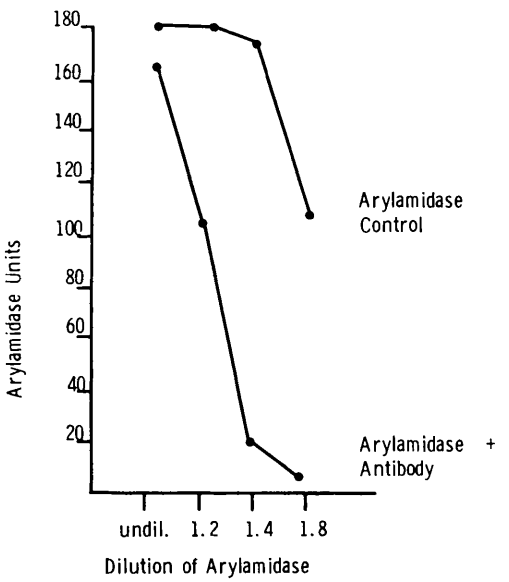

FIG. 1. Effect of antibody on arylamidase activity.

and substrate were mixed simultaneously and the rate of hydrolysis was determined. As a control, normal rabbit globulin replaced immune serum; also arylamidase control was used. The results are shown in Table I.

It can be seen that the antibody was inhibitory with and without preincubation. However, the antibody was a more effective inhibitor when enzyme and antibody were preincubated before the addition of substrate. It was also found that if the animals were given a second series of injections of arylamidase and antisera were collected this antibody produced a 90% inhibition of the arylamidase activity under the conditions of preincubation.

Studies were initiated to investigate the immunochemical specificity of antibodies to arylamidases. Antibodies were prepared to human liver arylamidase in the manner described above. It was found that the antibody to the liver enzyme inhibited the liver enzyme, but not the bacterial arylamidase, and antibody to the bacterial enzyme did not inhibit the liver enzyme. No cross reactions were observed in gel diffusion studies with these antibodies. However, some cross reactivity was shown between the antibody to *N. catarrhalis* arylamidase and an arylamidase found in *Neisseria perflava* in gel diffusion

TABLE I. Effect of Antibody on Arylamidase Activity.

	Test	Residual activity (%)
Preincubated 30 min then, substrate added	Normal rabbit γ-globulin + arylamidase	100
	Immune rabbit γ-globulin + arylamidase	40
Substrate added simultaneously	Normal rabbit γ-globulin + arylamidase + L-alanine βNA	100
	Immune rabbit γ-globulin + arylamidase + L-alanine βNA	55
	Normal rabbit globulin + L-alanine βNA	0
	Immune rabbit globulin + L-alanine βNA	0
	Purified arylamidase + L-alanine βNA	100

studies. A line of partial identity was shown to occur in gel diffusion with arylamidase from these two microorganisms, although no significant *in vitro* inhibition of *N. perflava* arylamidase was caused by antibody to *N. catarrhalis* arylamidase.

The nature of antibody inhibition of arylamidase was investigated by studying the effect of antibody on reaction velocity. Purified rabbit globulin was preincubated with arylamidase preparations for 30 min. The enzyme concentration in the reaction mixture was 19,800 units/ml. Velocities were determined at the following concentrations of L-alanine βNA shown in Table II. These data are presented in double reciprocal form in Fig. 2.

These results indicate that rabbit antibody to arylamidase, although highly specific and inhibitory, does not act as a competitive inhibitor. It is suggested that the inhibition is a result of conformational changes in the enzyme, or of steric hindrance of the interaction of enzyme and substrate. Liver arylamidase and bacterial arylamidase are apparently immunologically unrelated.

TABLE II. Substrate Concentrations and Reaction Velocities for the Determination of the Effect of Antibody on Michaelis Constant.

Substrate concentration (moles/liter)	Velocity[a] in presence of antibody	Velocity[a] without antibody
20×10^{-5}	11.6×10^{-3}	20×10^{-3}
15×10^{-5}	9.0×10^{-3}	14×10^{-3}
10×10^{-5}	7.0×10^{-3}	12×10^{-3}
5×10^{-5}	3.3×10^{-3}	6×10^{-3}
2.5×10^{-5}	1.8×10^{-3}	3×10^{-3}

[a] Expressed as change in absorbance/min; substrate concentration is that in the incubation mixture.

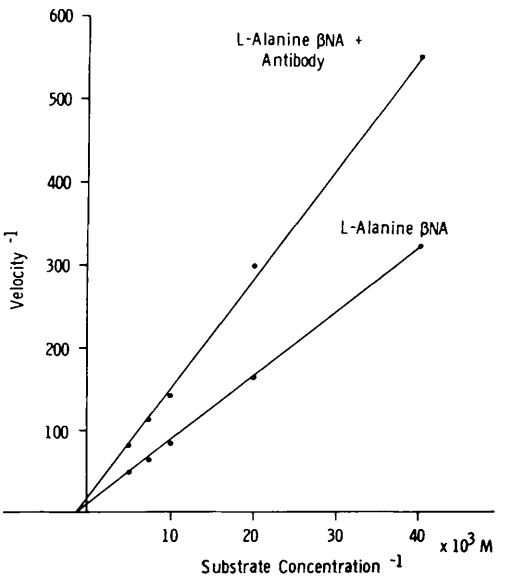

FIG. 2. The effect of antibody on the rate of L-alanine β NA hydrolysis.

Summary. A specific rabbit antibody to *N. catarrhalis* arylamidase was prepared. The antibody acted as a noncompetitive inhibitor of the hydrolysis of alanine-βNA by the bacterial arylamidase. Arylamidases from other sources are apparently immunologically unrelated to the bacterial enzyme.

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Inhibition of Friend Leukemia Virus Splenomegaly by an Acetylenic Carbamate (32819)

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Previous reports from our laboratories have described the synthesis (1–3), the oncolytic activity (4), and the anti-influenza activity (5) of a series of 1,1-diaryl-2-propynyl carbamates commonly called as a group the acetylenic carbamates. The antitumor studies indicated that the acetylenic carbamates were active against 16 of 21 transplantable tumors tested. The compounds were inactive against three types of ascites tumors indicating that the apparent activity was not due to contact cytotoxicity. Studies employing virus infecions in mice indicated that the acetylenic carbamates also possessed anti-influenza activity but were ineffective against infections in mice produced by polio, Semliki Forest, herpes, or pseudorabies viruses. Since anti-influenza studies had ranked 1-(4-fluorophenyl)-1-phenyl-2-propynyl *N*-cyclohexylcarbamate (FPPC) as the most interesting compound, FPPC was used in studies with Friend leukemia virus infections in DBA/2mice.

Materials and Methods. Plan of experiments. Mice were treated with FPPC either intraperitoneally by injection or orally by gavage. A standard dose schedule of drug administration at 24- and 4-hour preinfection and at 24- and 48-hour postinfection was used except when otherwise noted. Day of infection was considered day 0 and animals were autopsied for spleen removal and bled either 21 or 42 days after infection except where noted. Weight changes between start of drug treatment and time of autopsy were measured. Friend virus used in these studies came from a single lot prepared as the supernatant of a 10% spleen homogenate with a titer of about 10^5 infectious doses/ml. For each experiment a frozen ampul of virus was thawed, and diluted 1–100 in sterile media 199. A volume of 0.25 ml of diluted virus was inoculated intraperitoneally into DBA/2 mice (obtained from Jackson Laboratories, Bar Harbor, Maine) weighing between 14 and 16 gm.

Virus titrations. Spleens from treated and control mice 21 days after infection were homogenized with a Potter-Elvehjem type tissue homogenizer in media 199. The 10% spleen homogenate supernatants or harvested serums were diluted in 10-fold steps to 10^{-6} and then each dilution was injected into groups of 8 DBA/2 mice. After 21 days, recipient mice spleens were removed and weighed. Dilution of tissue was plotted against the log of spleen size and virus reduction was calculated as described by Chirigos (6).

Inhibition of splenomegaly. Since initial experiments indicated a decreased virus multiplication due to drug treatment, inhibition of splenomegaly was used in later studies to measure drug effect. The degree of inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Infected control spleen size—Treated spleen size}}{\text{Infected control spleen size—Normal spleen size}} \times 100.$$

Drug studied. Investigations (1–3) of the intramolecular reactions of acetylenic compounds led to the synthesis of a series of 1,1-diaryl-2-propynyl carbamates. The series of compounds was originally extended to include a study of structure–activity relationships based on the oncolytic activity. The combination of anti-influenza activity and