Lipoprotein Inhibitor of Newcastle Disease Virus from Chicken Lung

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A lipoprotein inhibitor of Newcastle disease virus was obtained from chicken lung tissue by means of dilute alkaline extraction procedures. The inhibitor was further purified by ammonium sulfate fractionation, isoelectric precipitation, and density gradient centrifugation. The purified lipoprotein inhibited active Newcastle disease virus hemagglutination at a concentration of 2.0 μg/ml which represented a 30-fold purification over the original extract. Infection of chicken embryo fibroblasts by Newcastle disease virus was also inhibited by the purified lipoprotein, the degree of inhibition depending upon the inhibitor-to-virus ratio. Chemical analysis of the purified inhibitor provided a composition of 72% lipid, 26% protein, and 3% carbohydrate, although some compositional variation was observed from one preparation to another. The chloroform-soluble lipids were shown to contain 40 to 50% phospholipid and 10 to 20% cholesterol; of the fatty acids recovered from the saponified lipoprotein, 39% was palmitic, 22% oleic, and 17% stearic. Careful analyses of large quantities of the inhibitor revealed a small (0.84%) but significant content of sialic acid. Removal of sialic acid from the lipoprotein by means of digestion with neuraminidase produced a sharp diminution in inhibitory properties. A delipidized form of the inhibitor was obtained by ether extraction, and this material produced a single broad band of precipitate in gel immunodiffusion tests.

The intensive past studies on the inhibitors of myxovirus hemagglutination and infectivity have focused primarily on those inhibitors present in sera (13, 17, 19). Among the most active of these inhibitors are those glycoproteins classified as Francis or alpha inhibitors, the activity of which is associated with the sialic acid portion of the molecule. Removal of the sialic acid with neuraminidase causes a loss of inhibitor activity. The beta and gamma class inhibitors of serum are resistant to neuraminidase and have generally been less well characterized than those of the Francis type.

In contrast to the ambitious investigations of myxovirus inhibitors on serum, tissue inhibitors, excepting those present on erythrocytes, have been largely ignored. Hirst (15) was the first to demonstrate that perfused ferret lung would first adsorb and then release influenza virus. A similar behavior of mouse, hamster, rat, guinea pig, rabbit, and chicken lung toward human and swine influenza virus, Newcastle disease virus, and mumps virus (34) has been described. It was an interesting observation that chicken lung did not adsorb Newcastle disease virus as well as the other influenza viruses and mumps virus.

Investigation of the myxovirus inhibitor in mouse lung has indicated that it is easily extracted by low molarity bicarbonate solutions (4, 23). No detailed chemical characterization of this solubilized inhibitor has yet been attempted; however, it was partially purified by differential centrifugation and Sephadex G-200 filtration, indicating its macromolecular nature. It was resistant to trypsin at 65 C for 30 min; however, it was sensitive to periodate and receptor-destroying enzyme, suggesting that it is a Francis-type inhibitor.

Laucikova (20) has described a lipoprotein inhibitor for influenza virus which she extracted from chicken embryo chorioallantoic membranes. The chemical composition of the inhibitor varied somewhat from one preparation to another but was approximately 55% protein, 18% lipid, and 2% carbohydrate. Extraction of the lipoprotein with ether destroyed the inhibitor, indicating its dependence upon lipid for its antiviral activity. Periodate treatment, exposure to receptor-destroying enzyme, or active virus also destroyed the activity of the inhibitor (21), supporting its classification as a sialic acid-dependent, Francis-type inhibitor.

The extraction of ox brain with a mixture of chloroform and methanol liberated an inhibitor
of influenza virus hemagglutination (31). It was described as a mucolipid consisting of as much as 20% sialic acid but no evidence was presented concerning the relation of the sialic acid to antiviral activity nor was the inhibitor described further in chemical terms.

The research reported here was initiated to determine whether inhibitors of Newcastle disease virus (NDV) infection and hemagglutination were present in chicken lungs. Since the lung is a primary tissue invaded by this virus, it was felt that the isolation of an inhibitor might be, simultaneously, an isolation of a natural receptor site for NDV. A unique lipoprotein inhibitor has been partially purified from chicken lungs and characterized in chemical terms. Its antiviral activity, as an inhibitor of viral hemagglutination and infectivity, has also been described.

MATERIALS AND METHODS

Unless stated to the contrary, all procedures were performed at 4 C.

Purification of lipoprotein inhibitor. Approximately 25 g of minced, washed chicken lungs were homogenized in 100 ml of 0.001 M NaHCO3 adjusted to pH 7.5 (26) with the aid of a Sorvall Omnimixer. After 10 min, this homogenate was diluted by the addition of 500 ml of cold bicarbonate solution. The diluted homogenate was clarified by centrifugation at 12,100 X g for 10 min. These conditions were employed for all future clarifications and for the collection of (NH4)2SO4 and isoelectric precipitates.

The clarified bicarbonate extract was adjusted to 20% saturation by the addition of solid, reagent-grade (NH4)2SO4, held for 24 hr and clarified by centrifugation. The supernatant was adjusted to 50% (NH4)2SO4 saturation by the addition of more ammonium sulfate and again held for 24 hr before being clarified by centrifugation. The precipitate was dissolved in 0.15 M phosphate-buffered saline (pH 7.2) which was 0.01 M with respect to ethylenediaminetetraacetic acid (EDTA). This solution was dialyzed against distilled water to remove the ammonium sulfate.

The dialyzed 20 to 50% ammonium sulfate fraction was adjusted to pH 5.0 by the dropwise addition of 1.0 M CH3COOH and held for 24 hr. The precipitate was collected by centrifugation and dissolved in 0.15 M phosphate-buffered saline containing 0.1 M EDTA (pH 7.5).

The redissolved isoelectric precipitate was placed on top of a 20 to 50% sucrose gradient prepared at room temperature with the aid of a Beckman density gradient former. The sucrose solution was prepared in 0.15 M phosphate-buffered saline (pH 7.5) containing 0.1 M EDTA. The gradient was subjected to 90,000 X g for 90 min in the SW25 head of a Spinco model L preparative ultracentrifuge. The top band (1.130 density) was collected, diluted by the addition of an equal amount of the buffer solution, and recentrifuged on another 20 to 50% sucrose gradient. The single band was collected and dialyzed against distilled water before chemical analysis. It was dialyzed against 0.15 M phosphate-buffered saline (pH 7.2) before hemagglutination inhibition (HAI) and plaque inhibition tests.

Chemical analyses. Protein determinations were performed by the method of Lowry et al. (24). Total hexose was determined by the method of Scott and Melvin (33) adapted to smaller volumes. A slight modification of the Elson and Morgan procedure (8) was used to quantitate hexosamine, and the thio-barbituric acid and diphenylamine methods of Warren (35) and Saifer and Siegel (32) were applied to the determination of sialic acid. The sum of hexose, hexosamine, and sialic acid is reported as total carbohydrate.

Total lipid was measured by the gravimetric procedure of Folch et al. (11). Twenty milliliters of chloroform-methanol (2:1) at room temperature was added to 20 mg of lyophilized inhibitor which was extracted for 1 hr with magnetic stirring before being filtered upon sintered glass. The insolubles on the glass filter were extracted with an additional 20 ml of solvent. Distilled water was added to the filtrate to just below a buret until the supernatant aqueous phase cleared. The lower chloroform phase was collected and dried completely under vacuum at room temperature before being weighed. Cholesterol was determined by the procedure of Zlatkis (37), and phosphorous, in the chloroform-methanol extract, was measured by Bartlett's method (3). Phosphorus was converted to phospholipid by assuming it represented 25% of the phospholipid. Fatty acids were determined as their methylated derivatives by gas chromatography (28) after saponification and petroleum ether extraction. For this analysis, 10 mg (dry weight) of lyophilized inhibitor sample was saponified with 2.5 ml of 2.5 M KOH in 85% methanol for 18 hr at 55 C. Unsonified materials were extracted three times with 5 ml of petroleum ether, and the aqueous phase was adjusted to pH 2.0 with 5 M HCl. Fatty acids were extracted by 2-5 ml applications of petroleum ether which were evaporated under vacuum and then dried over anhydrous Na2SO4. Methylation was accomplished by adding 1.0 ml of boron trifluoride-methanol reagent (25) to the dried product, followed by heat at 60 C for only 2 min. A petroleum ether extract of this mixture was washed with water before being evaporated to dryness. The methylated fatty acids were taken up in heptane and separated by a Barber-Colman, series 3,000 gas chromatograph operated with helium gas carrier at 190 C and a 20% ethylene glycol-succinate column on a gas pack (60 to 80 mesh). The flow rate was 80 ml/min, and the column effluent was analyzed by means of a hydrogen flame detector and paper strip recorder.

Virus and inhibitor titration, tissue culturing, and plaque inhibition. NDV was used throughout this study and was perpetuated in the chorioallantoic cavity of 9- or 10-day-old chick embryos, with harvesting 48 hr after inoculation.

Chick embryo fibroblast (CEF) cultures were prepared from 9- or 10-day-old embryos by the method of Hoskins (16) and grown in plastic tissue culture plates (60 by 15 mm) in Eagle's growth medium (7), con-
containing 200 units of penicillin and 200 μg of streptomycin per ml. Newborn calf serum was added to a final concentration of 10%. Monolayers were usually complete after 48 hr of incubation at 39 C under 5% CO₂.

For the plaque inhibition test, CEF cultures with complete monolayers were rinsed twice with 5 ml of cold (4 C) Hank's balanced salt solution (BSS) (14), lacking phenol red, and placed at 4 C. The lipoprotein inhibitor was dialyzed twofold in Hank's BSS containing either 96 or 180 plaque-forming units (PFU) of NDV per ml. These mixtures were added to the CEF cultures and held at 4 C for 1 hr to allow virus attachment. Thereafter, the excess fluid was removed and overlay agar was added. The overlay medium was composed of Eagle's medium with 5% calf serum and 0.2% Noble special agar. After 72 hr of incubation at 39 C, neutral red agar (12% Noble special agar, 0.25% lactalbumin hydrolysate, and 0.04% neutral red in Hank's BSS) was added, and plaques were counted. Triplicate plates were prepared for each concentration of inhibitor.

The hemagglutination (HA) titer of NDV was established as described by Hoskins (16). The titer of the hemagglutination inhibitor (HAI) was also determined by Hoskin's procedure (16). HAI titer is reported in terms of micrograms of inhibitor protein per milliliter which will inhibit 4 HA units of NDV at 4 C.

Neuraminidase digestion. Top band inhibitor from the sucrose gradient was dissolved in 0.08 M phosphate-buffered saline (pH 6.1) at a level of 4.4 mg/ml. Neuraminidase from Clostridium perfringens type IV (Sigma Chemical Co., St. Louis) was added to a final concentration of 287 μg/ml. The solution contained 0.02% sodium azide to retard microbial growth. This mixture was incubated at 37 C for 45 hr with constant shaking. Appropriate controls were employed in which lipoprotein was incubated under the assay conditions without the presence of neuraminidase. This digestion procedure is essentially that of Ada et al (1).

At the end of the digestion period, the samples were dialyzed against distilled water; the dialytes were then lyophilized and analyzed for sialic acid. The digested inhibitor and control were assayed for HAI activity after dialysis.

Preparation of lipoprotein antigen, immunization, and serological testing. The lipoprotein fraction resulting from the isoelectric precipitation at pH 5.0 was dissolved in 0.01 M phosphate buffer at pH 7.0 and adjusted to 2.0 mg of protein/ml. The HAI titer of this solution was 17.5 μg. Twenty-milliliters of this preparation was added to a carboxymethyl cellulose (CMC) column (70 by 1.9 cm) equilibrated with the same buffer but containing 0.02% NaN₃ as a preservative. The column was developed by batch elution at room temperature with 0.01 M phosphate buffers (pH 7.0 and 8.0), 0.20 M phosphate buffer (pH 9.0), and 2.0 M phosphate buffer (pH 9.5) containing 1.0 M NaCl. All buffers contained 0.02% NaN₃. The column was adjusted to a flow rate of 10.0 ml/hr, and fractions were collected with the aid of a Beckman gradient collector. The first fraction, which contained the inhibitor, was dialyzed against distilled water, lyophilized, and redissolved in 0.15 M phosphate buffer (pH 7.2). The inhibitor solution (330 μg of protein/ml) was injected intravenously into a rabbit with an irregular schedule over a period of 1 month, consuming approximately 2.0 mg of inhibitor before a capillary fluid precipitation test became positive. This antigen was also used in the immunodiffusion test.

A delipidized sample of the antigen was prepared by dissolving the lyophylized CMC, peak 1 product in 0.15 M NaCl to a final concentration of 330 μg of protein/ml. Four milliliters of this solution was added dropwise, with vigorous mixing, to 50 ml of an ethanol-diethyl ether solution (1:3) at −21 C (10). After 4 hr at this temperature, the precipitate which formed was collected by centrifugation (−21 C) and washed once with approximately 25 ml of diethyl ether (−21 C). The precipitate was dissolved in 0.13 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.6) containing 0.06 M sodium lauryl sulfate and was then dialyzed against this buffer lacking the detergent before use in the immunodiffusion experiment.

A third antigen used in the immunodiffusion test consisted of the top band sucrose gradient purified inhibitor.

The gel immunodiffusion experiment was performed essentially as previously described (2). The CMC peak 1 antigen was tested at 430 μg of protein per ml and lesser concentrations. The delipidized peak 1 antigen and the undelipidized top band gradient purified antigen were tested at 430 μg of protein per ml and 1.7 mg of protein per ml, respectively. The gel plates were cut with a commercially available cutter (Consolidated Laboratories, Chicago, Ill.) which spaced the center antisemum well 7.5 mm from the peripherally located antigen reservoirs. The serological reaction was developed at room temperature, and observations were made daily until the precipitation band diffused into a broad zone.

RESULTS

Table 1 illustrates the purification scheme used to isolate the lipoprotein inhibitor of NDV. The biological activity of all inhibitor fractions are reported as micrograms of inhibitor protein per milliliter which will inhibit 4 HA units of NDV at 4 C. The original, clarified bicarbonate homogenate of chicken lung had an HAI titer of 62.5 μg/ml. This value was quite constant from preparation to preparation, probably because each lot represented a pool of several lungs. The fraction precipitating between 20 and 50% ammonium sulfate saturation improved the HAI activity by approximately fourfold. This fraction had an HAI titer of 17.5 μg/ml. The 0 to 20% fraction generally had a titer of 95 μg/ml. Fractions recovered in the range 50 to 75% and greater than 75% ammonium sulfate saturation had titers in excess of 200 μg/ml. Ammonium sulfate fractionation removed hemoglobin from the inhibitor fraction.

Isoelectric precipitation at pH 5.0 produced a material with very good HAI activity (i.e.,
TABLE 1. Procedure for the purification of a lipoprotein inhibitor of Newcastle disease virus from chicken lung

| Step                                      | Hemagglutination inhibition titer (a) | Purification |
|-------------------------------------------|--------------------------------------|--------------|
| 1. Chicken lung homogenate 1:4 (w/v) in 0.001 M NaHCO₃ (pH 7.5) | 62.5 | 1.5 |
| 2. Twenty to 50% (NH₄)₂SO₄ precipitation | 17.5 | 3.6-fold |
| 3. Isoelectric precipitation (pH 5.0) with 1 M CH₃COOH | 9.0 | 7-fold |
| 4. Twenty to 50% sucrose gradient centrifugation, 90,000 × g | 2.0 | 31-fold |

* Titer expressed as the amount (micrograms) of protein inhibitor which will prevent hemagglutination by four hemagglutinating units of Newcastle disease virus.

HAI = 9 μg/ml). This product was poorly soluble in ordinary buffers unless stirred overnight in the cold. Sucrose banding of this material produced three bands (Fig. 1) the uppermost of which, at a density of 1.130, contained the inhibitor (HAI activity = 2.0 μg) in a 31-fold purification from the lung extract. The lowest band in the sucrose gradient appeared to consist of aggregated materials.

At each stage in the purification scheme, protein, carbohydrate, and lipid analyses were performed to provide insight into the chemical nature of the inhibitor. Table 2 presents a summary of these data, from which it can be seen that, as purification of the inhibitor progressed, the percentage of protein decreased and the percentage of lipid increased significantly. The purest preparation consisted of 3.6% carbohydrate, 26% protein, and 72% lipid, the sum of which account for the entire molecule. On the basis of the high lipid content, the molecule is referred to as the lipoprotein inhibitor hereafter.

More definite expressions of the carbohydrate and lipid content of the lipoprotein inhibitor are presented in Table 3. Of the carbohydrate, 1 to 2% was in the form of hexose, about 1% in the form of hexosamine, and 0.82 to 0.86% in the form of sialic acid. Chloroform-soluble lipids were analyzed for cholesterol and phospholipid which accounted for 10 to 20% of these lipids, respectively. Of the fatty acids recovered (Table 4), palmitic acid was the most abundant (39.3%), followed by oleic acid (22%) and stearic acid (17.6%). Six other fatty acids ranging from C₁₄ to C₂₀·₄ comprised the remaining 23% of the fatty acids.

The lipoprotein inhibitor is capable of preventing NDV infection of chicken embryo fibroblasts

FIG. 1. Sucrose gradient density stratification of lipoprotein hemagglutination inhibitor. Left tube: redissolved isoelectric precipitate (pH 5.0); 7 m centrifuged at 90,000 × g for 90 min on a Spinco model L preparative ultracentrifuge. Right tube: material taken from upper band of tube on the left and recentrifuged on a gradient as above. The upper band is the lipoprotein hemagglutination inhibitor.

TABLE 2. Chemical composition of the lipoprotein inhibitor of Newcastle disease virus

| Procedure                                      | Protein (%) | Carbohydrate (%) | Lipid (%) |
|-----------------------------------------------|-------------|------------------|-----------|
| Chicken lung homogenate in 0.001 M bicarbonate | 92          | 1                | Not done  |
| Saturation (20 to 50%) with (NH₄)₂SO₄         | 60          | 1.5              | 35        |
| Isoelectric pH 5.0 precipitation               | 42.5        | 1.5              | 54.2      |
| Sucrose gradient density, top band            | 26          | 3.6              | 72        |
Table 3. Chemical analysis of purified lipoprotein inhibitor of Newcastle disease virus

| Component                | Per cent |
|--------------------------|----------|
| Protein (Lowry)          | 20-30    |
| Hexose (orcinol)         | 1-2      |
| Hexosamine               | 0.8-1.0  |
| Sialic acid (diphenylamine) | 0.82-0.86 |
| Lipid                    | 72-74    |
| Cholesterol              | 10-20    |
| Phospholipid             | 40-50    |

* Determined as percentage of chloroform-soluble lipid.

Table 4. Fatty acid analysis of lipoprotein inhibitor

| Fatty acid carbon length | Common name | Per cent |
|-------------------------|-------------|----------|
| 14                      | Myristic    | 2.4      |
| 14:1*                   | Myristoleic | 1.0      |
| 16                      | Palmitic    | 39.3     |
| 16:1                    | Palmitoleic | 3.1      |
| 18                      | Stearic     | 17.6     |
| 18:1                    | Oleic       | 22.0     |
| 18:2                    | Linoleic    | 6.9      |
| 18:3                    | Linolenic   | 2.8      |
| 20:4                    | Arachidonic | 5.0      |

* Numbers expressed with colon indicate, by the first number, the number of carbons in the fatty acid and, by the latter number, the number of double bonds.

(Table 5). In the first experiment utilizing 96 PFU of virus, plaque reduction by as much as 64% was accomplished by 3.0 mg of inhibitor per ml. When the virus load was increased to 180 PFU, 3.0 mg of inhibitor per ml reduced the plaque count by only 14%, and lower concentrations were noninhibitory.

Digestion of the lipoprotein inhibitor with neuraminidase for 45 hr reduced its sialic acid content from 0.84 to 0.37% and lowered its HAI activity from 2 to 20 µg/ml, a 10-fold increase in activity. During the digestion period 40% of the sialic acid of the inhibitor was removed. Inhibitor incubated without enzyme did not change in HAI activity nor sialic acid content.

A typical elution pattern of the inhibitor fraction on carboxymethyl cellulose is presented in Fig. 2. The first elution peak corresponded with the void volume of the column and had an HAI titer of 9.0 µg of protein per ml. This represented a twofold purification over the isoelectric precipitation stage. Later elution peaks were not active against NDV virus. The CMC-purified fraction was chosen as the antigen for hyperimmunization of a rabbit.

Figure 3 presents the results of the Ouchterlony test after 24 hr. Precipitation was noted only when the delipidized antigen was tested against the antiserum. A rather broad band was formed. The untreated immunizing antigen failed to produce a precipitate even though it did react in the fluid.
DISCUSSION

The purification scheme for isolation of the lipoprotein inhibitor was devised by utilizing typical biochemical fractionation procedures and noting the improvement in HAI activity on a protein basis. Bicarbonate solutions are often used in the initial stages of cell membrane preparation (4, 23, 26, 34). Laucikova (20, 21) used bicarbonate extraction and isoelectric precipitation at pH 5.1 to separate an inhibitor of influenza virus HA from chick embryo chorioallantoic membrane extracts. Her inhibitor was composed of only 18% lipid and 55% protein and is thus quite dissimilar from the lipoprotein inhibitor described herein.

Our effort to utilize diethylaminoethyl (DEAE) cellulose in the purification scheme was unsuccessful; the lipoprotein inhibitor bound so tightly to the DEAE that it could not be eluted. Philipson et al. (30) also reported the loss of a lipoprotein inhibitor of enteroviruses upon ion-exchange chromatography. Blumenfeld (5) has reported complete retention of cell membrane sialoglycoproteins by DEAE, so this problem is not unique to this inhibitor. On the other hand, chromatography of the active material on CMC at neutral pH demonstrated that inhibitor activity was not retained on the column but eluted with the void volume. Some noninhibitory materials were retained on the column, and this procedure was employed in the preparation of the inhibitor antigen.

Because of the inability to use ion-exchange chromatography to great advantage, we attempted purification by sucrose gradient density centrifugation. Apparently, this method has not been used previously in the purification of viral inhibitors. Nevertheless, this method revealed that our isoelectrically precipitated product could be fractionated into three components, with approximately four- or fivefold improvement in purification. The lipoprotein inhibitor stratified at a density of 1.130.

The precise chemical composition of the NDV lipoprotein inhibitor varied somewhat from one preparation to another (Table 3). This is a typical problem in lipoprotein chemistry (10, 12, 20), the causes of which are not entirely known. Nevertheless, it is obvious that the inhibitor is not chemically similar to fowl immunoglobulins (22), neither is it chemically similar to fowl interferons (9) although it will prevent cell infection like the latter. It does not, in fact, have the same chemical composition as the influenza inhibitor from chicken chorioallantoic membrane described by Laucikova (20, 21). The plasma membrane from chick embryo fibroblast culture has a composition of 25% protein, 64 to 71% lipid, and 7 to 8% carbohydrate, very similar to that of the lipoprotein inhibitor (29). This suggests that the inhibitor is a portion of the cell membrane and adds significance to its description, for it could represent a natural receptor for the virus on chicken lung cells.

The major fatty acids present in the lipoprotein inhibitor are shown in Table 4 to be palmitic, oleic, stearic, linoleic, and arachidonic. These fatty acids have been reported to be in highest concentration in the influenza virus and in calf-kidney and chick-embryo host cells (18). It has been suggested that host lipids components are incorporated into the influenza virus particles at some time during their synthesis (12). Future work will attempt to determine whether lipid incorporated into Newcastle disease virus during infection resembles the lipid obtained as lipoprotein inhibitor from host tissue.

Newcastle disease, influenza, herpes simplex,
and vaccinia viruses have all been described as lipophilic viruses (27) based on their ability to adsorb to steroids including cholesterol, fatty acids such as palmitic and stearic acids, and various fatty acid derivatives. It is believed the attachment of these lipophilic viruses to cholesterol occurs via van der Waal's forces, whereas attachment to the fatty acids and their derivatives involves ionic forces (36). In some instances the adsorption is irreversible. Cholesterol, palmitic, stearic, and other fatty acids, all of which are known to serve as receptors for NDV (27), are present in the lipoprotein inhibitor prepared from chicken lung and may contribute to the inhibitory activities of this macromolecule.

All of the well-characterized hemagglutination inhibitors of myxoviruses contain sialic acid, upon which the basis for their biological activity resides (17, 19). Removal of 40% of the sialic acid in the lipoprotein inhibitor caused a 10-fold loss in its HAI activity, supporting its classification as a Francis type inhibitor.

The immunodiffusion test revealed a single band with the delipidized antigen only. Both of the lipid-bearing antigen preparations gave negative tests. The exact cause for this was not established. Hypothetically, this could result from the masking of antigen determinants in the protein by lipid so that they were unavailable to react with antibodies in the absence of any antilipid antibody formation, or simply the inability of the complete lipoprotein to diffuse in the aqueous menstruum owing to its hydrophobic characteristics. Although only a single precipitation band was detected even with the delipidized antigen, it was rather broad, indicative of antigenic impurity.

Important to the problem of whether this lipoprotein inhibitor is a "natural" receptor for the virus on chicken lung may be the observation that it will inhibit viral infectivity. Many inhibitors of myxovirus hemagglutination have been described (13, 17, 19) but few have been reported to retard infection by the virus. Ovine α₂-glycoprotein will inhibit both HA and plaque formation by NDV (6).

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