aortic acid mucopolysaccharides (AMPS) were studied. Rabbits fed a chow diet developed both a hypercholesterolemia and a hypertriglyceridemia in response to Medrol, and the already elevated cholesterol and triglyceride levels induced by cholesterol feeding were further elevated by Medrol. Despite the hyperlipemia, none of the Medrol-treated animals developed aortic atherosclerosis. Medrol had no significant effect upon the total aortic AMPS content in control animals but it did alter the aortic AMPS content and pattern of cholesterol-fed rabbits. The most significant effect was an increase in an AMPS of low sulfate content. Since all animals fed cholesterol developed some degree of pulmonary atherosclerosis, it is postulated that the antiatherogenic action of the hormone on the aorta is more closely related to alterations in the AMPS pattern than to changes in the pattern of circulating lipids.

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Method for Increasing the Diagnostic Capacity of the Complement Fixation Test in Some Respiratory Virus Infections (33384)

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It has been established, that the complement fixation (CF) test is of limited value for the diagnosis of respiratory virus infection in infancy and early childhood (1, 2). In both adeno- and parainfluenza virus infections, and especially in respiratory syncytial (RS) virus infection, not all of the diseased children from whom virus can be isolated, develop a significant antibody response as revealed by the CF test.

This might be explained in part by the fact that the 19 S (γM) immunoglobulin which is detected as the first antibody in many primary virus infections has little complement-fixing activity (3).

It was shown by Grubb (4) and Jones (5) that the polysaccharide dextran (6) reveals incomplete rhesus antibody when added in certain concentrations to the mixture of erythrocytes and serum. It had been shown some years earlier that bovine serum albumin is also a valuable diluent for rhesus typing reagents (7).

With the purpose of increasing the sensitivity of the diagnostic CF test for respiratory infections, the mixture of antigen, test serum, and complement has been supplemented with dextran and incubated overnight before the addition of the hemolytic system.

The CF titers obtained in tests performed with different dextran concentrations are reported in the present paper. Different respi-

ratory virus CF antigens have been employed. Sera from infants and children, most of them suffering from acute lower respiratory disease, have been examined.

Materials and Methods. The CF technique of Bradstreet and Taylor (8) performed with 0.1-ml volumes in Perspex plates was used in all tests, but with the following modification: A total volume of 0.4 ml of the mixture of complement (C), antigen, dextran, and serum was incubated overnight. In all tests here reported, the different dextran and salt concentrations (g/100 ml) refer to concentrations of dextran and salt in the 0.4-ml volume. Veronal buffered saline (VBS) (Oxoid Ltd., England) was used as a diluent. It must be stressed that the different sodium chloride (NaCl) concentrations given in Fig. 1 and Tables I-VI were obtained by employing a dilution of VBS giving these NaCl concentrations in the 0.4-ml volume. In all CF tests the sera examined were diluted in normal isotonic VBS and the hemolytic system was also made up in the same VBS.

Sera. These were obtained from children, 0–9 years of age, admitted to hospitals in Copenhagen mainly because of acute respiratory disease (9). The mean interval between the collection of the paired sera was 10 days. The sera were kept at —20° until examined. Seven immune sera from mice immunized against lymphocytic choriomeningitis virus (LCM) were also examined. These were from mice immunized a few weeks earlier with 3×10^3 LD_{50} of LCM virus either twice (sera days 16, 25, and 40) or three times (serum V 15). The other 3 immune sera were from the mothers of baby mice immunized with 3×10^3 LD_{50} of LCM virus shortly after birth. The mothers of baby mice immunized in this way are always infected by their litter and show presence of antibodies following the weanling period (10). All sera were inactivated for 30 min at 56° before being examined.

Antigens employed. The RS virus CF antigen and the parainfluenza type I and type III (P I and P III) antigens were the same as described in an earlier publication (9). The LCM antigen was kindly supplied by Dr. J. Hannover Larsen (11). All the antigens employed were titrated in checkerboard titrations (8), the respiratory virus antigens against pools of known positive sera from children and the LCM antigen against a known positive serum from an immune mouse.

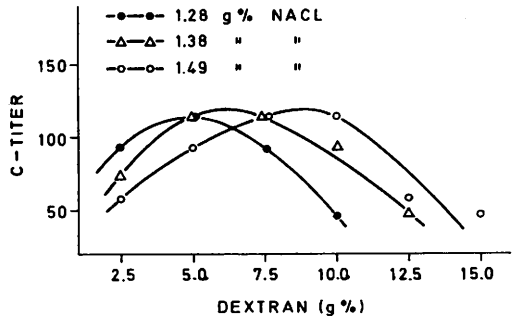

FIG. 1. C titers obtained in CF tests in three parallel experiments with constant NaCl concentrations and dextran concentrations ranging from 2.5 to 15.0 g/100 ml.

Dextran employed. Only dextran T 80 obtained from Pharmacia AB in Sweden and with a mean molecular weight of 80,000 was used. It was dissolved in VBS a few hours before use.

Results. In preliminary checkerboard titrations of C and hemolysin, C consumption was examined at different dextran and NaCl concentrations.

In titrations where only normal isotonic VBS was employed as the diluent, no hemolysis could be obtained when dextran was added in concentrations of 2.5, 5.0, 7.5, or 10.0% (g/100 ml).

In titrations with a constant dextran concentration of 5% and variable NaCl concentrations (ranging from 1.1 to 1.6%) the lowest C consumption was obtained with NaCl concentrations between 1.3 and 1.4%. In titrations with a constant dextran concentration of 10% and variable NaCl concentrations the lowest C consumption was obtained with NaCl concentrations above 1.4%.

In Fig. 1 is shown the C-consumption (C titer=reciprocal of C dilution giving 50% fixation) obtained in three different but parallel experiments each with a constant NaCl concentration (1.28, 1.38, or 1.49%) and dextran concentrations ranging from 2.5 to 15.0%. The C consumption examined is clear-

TABLE I. CF Tests with RS-virus Antigen.

Serum no.	Age of patient[a] y.	Age of patient[a] m.	Clinical diagnosis	Titers obtained: In VBS	Titers obtained: In VBS (1.38)[b] with 10% dextran
257 I	1	5	Acute tonsilitis	8	32
II				8	128
329 I	6	10	Pneumonitis	8	32
II				16	128
549 I	9	3	Bronchitis	8	64
II				<8	128
872 I	0	5	Pneumonitis	8	64
II				32	512
889 I	0	5	Pneumonitis	8	32
II				8	16
960 I	0	3	Pneumonitis	<8	<8
II				<8	8
1561 I	7	5	Pneumonitis	16	128
II				16	256
1600 I	1	3	Bronchial asthma	8	<8
II				8	<8
1657 I	1	5	Pneumonitis	16	64
II				16	128
2056 I	7	3	Pneumonitis	8	32
II				16	64
2084 I	4	4	Febrile convulsion	<8	64
II				8	64
2174 I	5	1	Pneumonitis	8	8
II				8	8
2275 I	5	8	Pneumonitis	16	128
II				16	128
2-68 I	0	11	Pneumonitis	<8	<8
II				8	32

[a] Years and months.

[b] VBS containing NaCl concentration of 1.38% employed.

ly dependent on the NaCl concentration employed. At a NaCl concentration of 1.49% the lowest C consumption is seen at dextran concentrations between 7.5 and 10.0%. At a NaCl concentration of 1.28% the lowest C consumption is seen at a dextran concentration around 5.0%.

It must be stressed, that all C titers given in Fig. 1 are values obtained at the same optimal hemolysin concentration (1:200).

In Tables I-IV examples are given of the value of incorporating dextran in CF tests with different respiratory virus antigens. As shown in Table I, the majority of titers obtained with dextran (CF-d titers) are clearly higher than the corresponding titers obtained in regular VBS (CF-v titers). The titers compared in the different tables are the results of parallel titrations of the sera in the different reaction mixtures investigated. Some other points are evident from examination of the CF titers shown in Table I. The majority of the CF-v titers changes occurring in the course of the disease are reproduced but at a

TABLE II. CF Tests with P I (Sendai) Antigen.

Serum no.	Age of patient[a] y.	Age of patient[a] m.	Clinical diagnosis	Titers obtained: VBS	Titers obtained: VBS (1.38)[b] with 7.5% dextran
593 I	1	9	Atypical pneumonitis	<8	8
II				8	32
872 I	0	5	Pneumonitis	16	64
II				16	32
1180 I	0	1	Pneumonitis	<8	8
II				<8	8
1222 I	6	0	Acute pharyngitis	8	16
II				16	128
1721 I	5	2	Pneumonitis	16	128
II				16	64
1952 I	3	5	Pseudocroup	8	16
II				8	16
2084 I	4	4	Febrile convulsion	16	32
II				16	32
2275 I	5	8	Pneumonitis	16	128
II				16	64

[a] Years and months.

[b] VBS containing NaCl concentration of 1.38% employed.

higher level in the corresponding CF-d titers. Furthermore a few paired sera showing no significant CF-v titer increase do show significant CF-d titer increases. It is also evident that the differences between the CF-d and the CF-v titers found do not depend on the age of the patient, but too few sera in the limited age group 0–9 years have as yet been examined for a final conclusion to be made. It must be stressed that the sera listed in Tables I–IV have not been selected from a large number of sera examined, but do represent in general sera from children with acute respiratory disease. The results with P I and P III antigens in Tables II and IV showed essentially the same differences between CF-d and CF-v titers found in CF tests with the RS antigen (Table I).

TABLE III. CF Tests with P I Antigen in Dextran with Different NaCl Concentrations (1.08, 1.38, and 1.48%).

Serum no.	Titers obtained: VBS (1.08) dextran 7.5%	VBS (1.38) dextran 7.5%	VBS (1.49) dextran 7.5%
1222 I	ND	16	ND
II	≧256	128	≧256
1721 I	≧256	128	≧256
II	128	64	128
2084 I	64	32	64
II	64	32	64
2275 I	256	128	64
II	128	64	64

Table III presents the CF-d titers with P I antigen on a few paired sera, using a constant dextran concentration but different NaCl concentrations in parallel titrations. Except for the possibly higher titers in serums 1222 II and 1721 I, no significant difference in titers was found with different NaCl concentrations (1.08, 1.38, and 1.49%).

In Table V the results of CF tests with LCM antigen and sera from mice immunized with LCM virus by intraperitoneal injections and sera from mothers of immunized baby mice are presented. Of the CF-d titers obtained with 5.0 and 7.5% dextran and 1.49%

TABLE IV. CF Tests with P III Antigen.

Serum no.	Age of patient[a] y.	m.	Clinical diagnosis	Titers obtained VBS	VBS (1.38)[b] with 10% dextran
859 I	0	9	Pneumonitis	8	16
II				8	64
889 I	0	5	Pneumonitis	8	16
II				8	8
960 I	0	3	Pneumonitis	<8	8
II				<8	8
1600 I	1	3	Acute bronchitis	8	32
II				8	8
1821 I	1	9	Acute bronchitis	8	16
II				8	32

[a] Years and months.
[b] VBS containing NaCl concentration of 1.38% employed.

NaCl only one titer (serum 16) is significantly different from the corresponding CF-v titers. With 10% dextran almost all sera show significantly higher titers than the CF-v titers. However, as was the case with the titers obtained with P I antigen (Table III), no significant differences were found at different NaCl concentrations but with constant dextran concentration (Table V). The effect of heating the same immune sera to 65° for 1 hr is shown in Table VI. The CF-d titers obtained after heating did not differ from the corresponding CF-v titers. This is best seen with sera days 16, 25, and 40. The antibodies measured in immune mouse sera by incorporation of dextran, are apparently not more thermostable at 65° than antibodies detected by regular CF tests. A similar lack of thermostability of CF antibodies to RS virus has been found in a few human sera and in a few guinea pig antisera titrated in dextran.

Discussion. In addition to bovine serum albumin and dextran other high molecular compounds like gelatin and polyvinylpyrrolidone have been found useful in the detection of incomplete antibodies to blood group antigens (7).

In experimental infections with influenza virus in laboratory animals a certain concentration of normal serum has been shown to enhance the activity of neutralizing antisera (12, 13).

In several checkerboard titrations of the antigens employed, an increase in titer of the antiserum employed was seen more often than an increase of titer of antigen in the

TABLE V. Tests with LCM Antigen in Different Dextran (D) Concentrations.

Immune serum	Titers obtained VBS —D	VBS (1.49) 5.0% D	VBS (1.49) 7.5% D	VBS (1.49) 10.0% D	VBS (1.38) 7.5% D
Day 16	64	128	256	512	256
Day 25	64	128	128	512	256
Day 40	64	128	128	256	256
V 15	64	64	128	128	128
Imm. m.9/12	64	128	128	256	128
Imm. m.2/2	128	256	256	1024	512
Imm. m.20/2	64	128	128	512	256

TABLE VI. CF Tests with LCM Antigen after Extra Heating of Sera.

Immune serum	Titers obtained after heating to 65° for 60 min			
	VBS —D	VBS (1.38) 5.0% D	VBS (1.38) 7.5% D	VBS (1.49) 10.0% D
Day 16	64	64	128	ND
Day 25	64	32	32	32
Day 40	16	32	32	16
V 15	<16	16	<16	16
Imm. m.9/12	<16	16	<16	<16
Imm. m.2/2	<16	16	16	<16
Imm. m.20/2	<16	16	16	<16

titrations where dextran was incorporated. It seems probable, that the dextran in some way increased the activity of the antibodies in the sera examined from the infants and children with acute respiratory disease. Does the added dextran uncover a new kind of antibody or does it only make detection of known antibody easier? Since most of the results showed that the CF-d titers follow the CF-v titers closely but at a higher level, an affirmative answer to the latter part of the question seems more probable.

When the CF titers obtained in reaction mixtures with different salt and dextran concentrations were evaluated and compared, proper consideration was paid to the absolute amounts of C employed. C and hemolysin titrations, which were always repeated in the presence of antigen, sometimes showed that a smaller absolute amount of C could be employed in the presence than in the absence of dextran. With RS antigen (Table I) the CF-d titers were obtained using a smaller absolute amount of C than in the case of the CF-v titers. With the P I antigen (Table II) the CF-d and the CF-v titers were obtained using the same absolute amount of C. Therefore different amounts of C employed cannot explain the higher titers obtained by employing dextran in the reaction mixture.

In various experimental virus infections in laboratory animals the early appearing γM antibody has shown lower avidity, lower CF activity and has been more thermolabile that the γG antibody appearing later (3). Thus it is possible that the detection of early appearing γM antibody by CF tests is made easier by the addition of dextran.

It should be noted that dextran has been shown to be an antigen related to certain pneumococcus and salmonella species (6). This antigenic relationship might possibly limit the use of dextran in CF tests with sera from older children and adults with a broader antibody spectrum.

Summary. The effect of adding dextran T 80 (av mol. wt. 80,000) in certain concentrations to the mixture of antigen, complement (C) and serum in the diagnostic CF test for RS and parainfluenza (P) virus infections has been investigated. Preliminary experiments showed that the diluent employed (Veronal buffered saline, VBS) had to be used with hypertonic salt concentrations to prevent C consumption from increasing when dextran was added to the test system. Sera from infants and children with respiratory infections were titrated in parallel CF tests using either regular VBS without dextran (CF-v) or hypertonic VBS supplemented with dextran (CF-d). In the majority of sera examined the CF-d titers were significantly higher than the corresponding CF-v titers. Furthermore, using dextran, a rise in titer in the second of a pair of sera was obtained, which was not found in regular CF tests without dextran. Addition of dextran was also able to increase the CF titers in tests with LCM antigen and immune sera from mice.

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Persistence of West Nile Virus in L-929 Mouse Fibroblasts* (33385)

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It has been demonstrated that under certain conditions a variety of mammalian cell cultures can be chronically infected with viruses and that both cells and infecting virus can replicate simultaneously (1–3). Chronic infections have been induced in the presence of both specific antiviral antibodies and nonspecific antiviral substances as well as by nutritional deficiencies (3). In only a few instances have chronically-infected cell cultures been established when routine cultural methods were employed (3). The present paper reports the establishment of a chronic infection in L-929 mouse fibroblasts (4) with a virulent group B arbovirus, West Nile virus (5), using routine cultural methods.

Materials and Methods. Cell cultures. The L-929 mouse fibroblasts were obtained from Microbiological Associates, Inc. Stock cell cultures and infected cell cultures were grown in a modified Eagle's minimum essential medium (6)[1] prepared in Hanks' balanced salt solution[1] and supplemented with 1 m*M* sodium pyruvate,[1] 0.1 m*M* nonessential amino acids,[1] 50 units of penicillin/ml, 100 μg of streptomycin/ml and 5% calf serum[1] (H-MEM). All cell cultures were grown in 12 ml of medium in tightly sealed 250-ml square glass bottles incubated at 36°. Medium was routinely replaced at 3- or 4-day intervals. Subcultures were initiated by replacement of H-MEM with 0.2% trypsin.[2] After cells were released from the glass, they were centrifuged, trypsin was decanted, and cells were resuspended in fresh H-MEM.

Virus. The West Nile virus (WNV) used in this study was obtained from American Type Culture Collection as a 10% mouse brain suspension (twenty-fifth passage) in normal mouse serum. Stock virus [Arkansas Mouse Passage 8 (AMP 8)] was prepared as supernatant fluid of infected mouse brain homogenate in sufficient 50% normal rabbit serum in saline to make a 10% brain suspension (w/v). Sealed glass ampuls of AMP 8 were shell frozen and stored in a CO_2 box. The titer of WNV in AMP 8 was determined by intracerebral inoculation of 3-week-old Swiss mice. Tenfold dilutions were made in H-MEM and 0.03 ml of each dilution was injected into each of 5 mice. The LD_{50} titers were calculated by the method of Reed and Muench (7) and ranged from $10^{-7.2}$ to $10^{-7.7}$ when titrated five times over a period of 14 months.

Initial infection of cell cultures with WNV. After removal of medium from normal

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[1] Microbiological Associates, Inc.

[2] Difco certified, 1:250.