the chest provides a simple and reproducible method of initiating primary cell strains from rabbits.

Authors' note. While this article was in press we became aware of two articles which supported the possibility that peripheral blood long-term cell strains arise by needle biopsy contamination. These reports were based on experiments with guinea pigs (Ross, R., and Lillywhite, J. W., *Lab. Invest.* **14**, 1568 (1965)) and chickens (Rangan, S. R. S., *Exp. Cell Res.* **46**, 477 (1967)), and thus support and extend our own observations.

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Reaction of Infectious Mononucleosis Sera with Cell Cultures Infected by Newcastle Disease Virus* (32696)

ARTHUR FRIEDMAN,[1] ALMEN L. BARRON, AND FELIX MILGROM
Department of Bacteriology and Immunology, School of Medicine, State University of New York at Buffalo, Buffalo

It has been shown (1–3) that human red blood cells (RBC's) treated by Newcastle disease virus (NDV) are agglutinated by sera of patients suffering from infectious mononucleosis (IM). It was decided to investigate whether alteration of cells other than RBC by NDV would result in their reaction with IM sera. Cell cultures infected with NDV were selected for this investigation. To detect the reaction of IM sera with the surface of the cell cultures, altered as a result of infection by NDV, the mixed agglutination (MA) procedure was used (4).

Materials and Methods. Viruses. The VIC strain of NDV was obtained from Dr. D. T. Karzon, Virology Laboratory, Children's Hospital, Buffalo. The B1 strain of NDV was received from the American Type Culture Collection. Viruses were propagated in the allantoic cavity of the chick embryo. For mixed agglutination experiments, VIC strain was passaged in human cell cultures of HEp-2 line.

Cell cultures. The HEp-2 line passaged routinely in this laboratory was purchased originally from Flow Laboratories, Rockville, Maryland. The growth medium consisted of Eagle's basal medium (BME), and 10% newborn calf serum (Grand Island Biological Company) in a base of Hanks' balanced salt solution (BSS). When cultures were inoculated with virus, the growth medium was replaced with maintenance medium composed of Eagle's minimal essential medium (MEM), and 3% calf serum in a base of Earle's salt solution. Primary cell cultures of Rhesus monkey kidney were grown in a medium consisting of 0.5% lactalbumin hydrolysate (Nutri-

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[1] Present address: Merck Institute for Therapeutic Research, Virus and Cell Biology Division, West Point, Pennsylvania.

tional Biochemicals Corporation), and 2% newborn calf serum in a base of Hanks' BSS as previously described (5). The maintenance medium was Eagle's MEM and 3% newborn calf serum. Primary guinea pig kidney cultures were prepared following the procedure used for monkey kidney cultures. The cells were grown in medium 199 and 20% fetal calf serum. The maintenance medium consisted of Eagle's MEM and 3% newborn calf serum. Chick embryo cultures were prepared from 10-day-old embryos which were treated with trypsin after decapitation (6). The growth medium was composed of 0.5% lactalbumin hydrolysate, and 4% newborn calf serum in a base of Hanks' BSS. The maintenance medium was 50% bovine amniotic fluid (Hyland Laboratories), 4% newborn calf serum, and 46% Hanks' BSS.

Penicillin at a concentration of 200 units/ml and streptomycin at a concentration of 200 μg/ml were incorporated in all media.

Human sera. Sera from IM patients were received from the Student Health Service, State University of New York at Buffalo, through the courtesy of Drs. P. F. Hoffman and M. L. Kunz. Some IM sera were also generously supplied by Dr. O. Tönder, Department of Microbiology, University of Bergen, Norway. Sera from patients with various viral diseases were obtained from the Erie County Virology Laboratory. Sera of patients suffering from rheumatoid arthritis, multiple myeloma, leukemia, syphilis, multiple sclerosis, lupus erythematosus, and also cord sera and sera from healthy blood donors were obtained from the Buffalo General Hospital.

Mixed agglutination. The MA procedure with cell cultures was based on methods previously described (4,7). (*a*) *Treatment of sheep RBC.* A mixture of 10 volumes of a 1% suspension of sheep RBC and one volume of a 1:10 dilution (185 units/ml) of receptor destroying enzyme (RDE, Sigma Chemical Company, St. Louis), was incubated for 1 hour at 37°C. A saline solution containing calcium (8) was used as diluent during the treatment. After the incubation, the RBC's were washed three times and resuspended in 0.15 *M* saline to 4%. When the RDE-treated RBC's were added to cell cultures infected with NDV in a conventional viral hemadsorption test, negative results were obtained. (*b*) *Indicator systems.* (*i*) Multispecific indicator. The RDE-treated sheep RBC's were mixed with an equal volume of a 1:40 (weakly agglutinating) dilution of human or chimpanzee anti-sheep RBC serum, and the mixture was incubated at room temperature for 45 minutes. The RBC's were washed three times and resuspended to 2% in saline. An equal volume of a 1:10 diluted goat antiserum to human serum was added. The mixture was incubated at room temperature for 45 minutes. The RBC's were washed three times in saline, dispersed, and resuspended in a medium composed of 0.5% lactalbumin hydrolysate (Nutritional Biochemicals Corporation) in Hanks' BSS (LH). (*ii*) IgM indicator consisted of RDE-treated sheep RBC's sensitized with human or chimpanzee anti-sheep RBC serum and agglutinated by goat antiserum to human IgM (Hyland). A procedure similar to that described for the multispecific indicator was followed. (*iii*) IgG indicator was similar to the two other indicators except that a goat antiserum to human IgG (Hyland) was used. (*c*) *Test procedure.* The HEp-2 cell cultures were used for most MA experiments. Cultures were inoculated with approximately 1000 TCD_{50} of NDV per tube and were incubated at 36°C for 48 hours. At this time, viral cytopathic effect (CPE) was marked, and when such cultures were tested with human RBC, a positive viral hemadsorption test was observed. Infected cell cultures were washed twice with LH medium warmed to 37°C. Various dilutions of the serum, to be tested, prepared in LH medium were added in 0.5-ml volumes to the cell cultures, and the tubes were incubated for 3 hours at room temperature. The serum was removed and the cell cultures were washed three times with LH medium. Thereafter, 0.5 ml of the indicator was added to each tube and the cultures were incubated for 1 hour at room temperature. The tubes were gently shaken and examined microscopically for adherence of indicator cells to the monolayer. The scoring system was similar to that previously described for detection of

measles antibody by mixed agglutination (7). The reciprocal of the highest dilution of serum giving a definite positive reaction was taken as the MA titer. Infected cell cultures treated with LH medium alone and uninfected cell cultures were included in every experiment as controls. Adherence of the indicator was not encountered in control cultures.

Other procedures. The other procedures used in this study, heterophile test, agglutination of NDV-modified RBC, and diffusion in agar gel, are described in detail elsewhere (9).

Results. Preliminary experiments were performed with HEp-2 cell cultures infected with VIC strain of NDV and the multispecific indicator. Positive results were obtained with some IM sera; however, titers were rather low and no clear-cut distinction could be made between these sera and normal sera. For this reason, it did not appear that the multispecific indicator would be satisfactory for this study.

It has been established that antibodies participating in the agglutination of NDV-modified RBC's are of the IgM type (10). Accordingly, MA experiments wer performed employing the IgM indicator. As a sample protocol shows (Table I), high MA titers were

TABLE I. MA Test with IM Sera; IgM Indicator Was Used.

Serum dilution	IM Serum 971	1061	1141	1201	1291	Five control sera
	HEp-2 cell cultures infected with NDV					
1:10	4+	4+	4+	4+	4+	—
1:40	4+	4+	4+	4+	4+	—
1:160	3+	4+	4+	2+	3+	—
1:640	2+	2+	3+	—	1+	—
1:2560	—	—	2+	—	—	—
1:10,240	—	—	—	—	—	—
	Uninfected HEp-2 cultures					
10	—	—	—	—	—	—
40	—	—	—	—	—	—

found with IM sera and negative results were obtained with control sera. In another experiment, IM sera were treated with 2-mercaptoethanol; this resulted in complete disappearance of their activity in MA. The heterophile test and the test based on agglutination of NDV-modified RBC were also negative with IM sera treated with mercaptoethanol.

The MA test was also performed with the IgG indicator. Positive results were obtained with some IM sera, but the antibody titers were considerably lower than those obtained with IgM indicator. Results in the MA test with IgG indicator were hardly affected by mercaptoethanol treatment of IM sera. The IgM indicator was employed in the remainder of experiments.

Sera from individuals suffering from a variety of diseases other than IM and from healthy blood donors were also examined in the MA test. Of 98 sera (Table II) from patients with other illnesses, only 8 (8.2%) yielded positive results and the titers did not exceed 40. Sera from 7 patients with infectious hepatitis were negative in the MA test, which was of interest because positive results have been reported with infectious hepatitis sera according to the procedure of agglutination of NDV-modified RBC's (3). Among the 12 sera of blood donors, two had a titer of 40, and negative results were obtained with 6 cord sera. Thus, none of the 116 control sera tested had a titer above 40 in the MA test. This is in contrast to the results obtained with the 65 IM sera, of which 35 (53.8%) had titers of 160 or greater in this test.

The relationship of titers obtained by heterophile test, agglutination of NDV-modified

TABLE II. MA Test with Sera from IM Patients, Patients with Various Diseases, and from Normal Individuals.

Clinical category	No. sera with MA titer of:				
	≤10	40	160	≥640	Total
Infectious mononucleosis	12	18	20	15	65
Infectious hepatitis	7	0	0	0	7
Rheumatoid arthritis	10	2	0	0	12
Multiple myeloma	1	0	0	0	1
Leukemia	3	0	0	0	3
Syphilis	5	0	0	0	5
Multiple sclerosis	10	2	0	0	12
Lupus erythematosus	2	0	0	0	2
Suspected virus infection	52	4	0	0	56
Blood donor	10	2	0	0	12
Cord sera	6	0	0	0	6
Total	106	10	0	0	116

RBC's, and MA test was investigated. To verify the heterophile test, sera were absorbed with guinea pig kidney (Baltimore Biological Laboratories). In no case was the agglutination titer for sheep RBC affected by absorption. The results are summarized in Table III. A rank correlation coefficient (11) of +0.77 was obtained when the agglutination of NDV-modified RBC's was compared with the MA test. This indicated a strong positive correlation for titers obtained by the two procedures. On the other hand, the rank correlation coefficient was +0.06 when the heterophile test was compared with the MA

TABLE III. Comparison of Titers Obtained by Heterophile Test, Agglutination of NDV-Modified RBC's, and MA Test with Sera from 65 IM Patients.

A. Heterophile titer

MA titer	80	160	320	640	1280	Total
<10	0	3	0	2	1	6
10	0	1	2	1	2	6
40	2	7	4	2	3	18
160	4	7	4	2	3	20
640	3	1	2	3	2	11
2560	0	0	1	1	2	4
Total	9	19	13	11	13	65

B. Titer agglutination of NDV-modified RBC's

MA titer	≤10	20	40	80	160	320	640	1280	Total
<10	5	1	0	0	0	0	0	0	6
10	3	0	1	2	0	0	0	0	6
40	3	6	6	2	1	0	0	0	18
160	3	2	2	7	3	3	0	0	20
640	0	0	1	0	2	4	2	2	11
2560	0	0	0	0	1	0	0	3	4
Total	14	9	10	11	7	7	2	5	65

test, which indicated a very slight correlation.

For some IM patients several serum specimens were collected at various times during the course of the disease. No information was available as to the exact date of onset of illness. These sera were examined in the three tests mentioned above (Table IV). Two types of changes in antibody status were observed. A rise in antibody titer was demonstrated in all three tests for 521 and 551. A decline in titer after a considerable period of time was shown for 861 and 1141, again in all three tests.

TABLE IV. Titers of Sequential Serum Specimens from IM Cases in Heterophile Test, Agglutination of NDV-Modified RBC's, and MA Test.

IM serum	Titer		
	Hetero-phile	NDV-modified RBC's	MA
521			
11-5-63	<10	<10	10
11-11-63	160	20	80
12-11-63	160	40	≥320
551			
12-2-63	160	40	40
1-6-64	1280	640	640
861			
3-5-65	640	160	10,240
4-9-65	640	160	2560
10-6-66	<10	20	40
1141			
3-16-66	320	2560	2560
4-28-66	160	2560	640
10-6-66	<10	40	40

Absorption experiments were performed to obtain additional information on the relationship of antibodies detected by the agglutination of NDV-modified RBC's and the MA test. Absorption of IM sera with sheep RBC's removed only the heterophile antibodies, whereas absorption with NDV-modified RBC's removed antibodies detectable by the agglutination of NDV-modified RBC's and by the MA test. Absorption with untreated human RBC failed to affect any of the three tests.

In one experiment, IM sera were incubated with NDV-infected HEp-2 cell cultures and, after washing, NDV-modified RBC's were added to the cultures. In this test, adherence of erythrocytes to the cell cultures was also observed, although the antibody titers obtained were lower than titers recorded in the MA test. In this experiment, the antibodies of IM sera apparently provided bridges connecting the cell culture and RBC's. In other words, the experiment showed that the same antibody molecule can combine with NDV-infected cell cultures and with NDV-modified RBC's.

Other investigators (1,12) have shown that erythrocytes from chicken and guinea pigs could also be modified by NDV and subsequently agglutinated by IM sera. It was therefore interesting to determine if NDV-infected cell cultures of species other than man would react with IM sera. Primary cell cultures of chick embryo, Rhesus monkey kidney, and guinea pig kidney were tested in addition to HEp-2 cells. CPE was observed in chick embryo and monkey kidney cultures. The viral hemadsorption test was positive when human erythrocytes were added to the infected cell cultures. On the other hand, guinea pig kidney cultures did not demonstrate any CPE, and the hemadsorption test was negative. As expected, IM sera gave positive results in the MA test with infected chick embryo and monkey kidney cell cultures, but not with guinea pig kidney cell cultures. Parallel results were not obtained when the erythrocytes of these species were treated with NDV. Monkey RBC's were not agglutinated and could not be modified for reaction with IM sera whereas chicken and guinea pig RBC, which were agglutinated by the virus, could be modified.

All of the above experiments were performed with the VIC strain of NDV. Since it has been reported that not all strains of NDV could be used for modification of RBC's (13), MA tests were performed with the B1 strain of NDV. While VIC strain could be readily passaged in HEp-2 cultures, it was found that B1 could not be passaged with any degree of success, and it was necessary to inoculate HEp-2 cultures with allantoic fluid infected with B1 virus. In such cultures, a cytopathic effect and positive viral hemad-

sorption reaction was obtained. Essentially negative results were found in the MA test when B1-infected HEp-2 cultures were reacted with IM serum. It was shown in control experiments that rabbit anti-NDV serum prepared against VIC strain would react in the MA test with cultures infected by either VIC or B1. This experiment required the use of an indicator designed to demonstrate binding of rabbit antibodies to cell cultures.

In another study (9) we have demonstrated that IM sera form reaction lines in a gel diffusion test when diffused against NDV preparations. Positive results were obtained with VIC strain of NDV but not with B1 strain or other myxoviruses. The 65 IM sera listed in Table II were examined for activity in the gel diffusion test, and the results are compared with titers obtained by MA. As can be seen in Table V, very good

TABLE V. Comparison of MA Titers and Reactions in Gel Diffusion with Sera from 65 IM Patients.

Gel diffusion	MA titer					
	<10	40	160	640	2560	Total
Positive	0	8	20	11	4	43
Negative	12	10	0	0	0	22

correlation was obtained between the two tests. Thirty-five sera with titers of 160 or greater in the MA test were all positive by gel diffusion. At the other end of the scale, sera with low MA titers were also negative in gel diffusion.

Discussion. The major objective of this study was to learn whether the action of Newcastle disease virus in conditioning the reactivity with infectious mononucleosis sera is restricted to RBC or can be demonstrated with other cells. For this purpose cell cultures of various species origin were infected with NDV and studied for their reactions with IM sera. Positive results were obtained by the mixed agglutination procedure which demonstrated reaction between infected cell cultures and IM sera. Sera from healthy individuals or individuals with other diseases did not react with NDV-infected cell cultures. From this study it became apparent that the association of NDV with IM sera is not due to a peculiarity of RBC and that other cells can be made reactive with IM sera by NDV.

The results in the MA test correlated well with the test employing NDV-modified RBC's. Evidence was presented that the same antibody participated in both tests. In view of the fact that others have shown that the antibody active in the test employing NDV-modified RBC's belongs to the IgM class (10), it was not surprising that best results in the MA test were obtained with an indicator system specifically designed to detect the binding of human IgM to cell cultures.

In considering the agglutination of NDV-modified RBC's, two mechanisms have been contemplated. One of these proposes that IM sera combine with a normal erythrocyte antigen exposed by the virus (14). The alternative view is that the RBC's acquire an antigen on their surface from the infected allantoic fluid (1). The data obtained by the MA test and also by gel diffusion (9) obviate the requirement for RBC's and thus favor the second explanation.

In the agglutination of NDV-modified RBC's the VIC strain of NDV was shown to be active, but, negative results were obtained with B1 strain (13). Similar results were obtained in this study in the MA test and in another study with gel diffusion (9). The speculation can be entertained that VIC strain has two sites, designated *N* (Newcastle) and *M* (Mononucleosis). Strain B1, on the other hand, has only *N* site. Agglutination or infection of cells by VIC results in the appearance of *N* and *M* on the cell surface. The IM sera which contain *M* antibodies can thus react with these cells. In extending this speculation it is attractive to consider that *M* antibodies in IM sera appear in response to infection by the causative agent of infectious mononucleosis. The VIC strain of NDV and the IM agent could share *M*, similarly as has been observed in other instances among microorganisms, even when they are biologically unrelated.

Summary. Reaction of sera from patients with infectious mononucleosis (IM) with cell cultures infected by NDV was demonstrated by mixed agglutination (MA). The indicator

system used in MA was specific for human IgM. A high incidence of positive results was obtained for IM sera in contrast to a low incidence in sera of patients suffering from other diseases and sera of healthy individuals. Good correlation was obtained between results in MA and tests with agglutination of NDV-modified RBC's. Positive results were obtained with IM sera using the VIC strain of NDV in MA, but not with the B1 strain.

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Failure of Methimazole to Affect Peripheral Thyroid Indices in Man* (32697)

JEROME J. BALLANTINE,[1] AND LEO OLINER
Metabolic Research Laboratory, Veterans Administration Hospital, and the Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana

Thiouracil compounds have been extensively utilized both experimentally and therapeutically. Their antithyroid action at the level of the thyroid gland is well known (1). Extrathyroidal effects of these substances have been reported (2–4). Propylthiouracil (PTU) has been shown to produce a slowing of the plasma loss of radioactivity following the injection of ^{131}I-labeled L-thyroxine in man (2,3,5), with no effect on the thyroxine-binding serum protein carriers (3,5). In these studies a decrease in urinary and an increase in fecal radioactivity was noted (2,3). The excretory pattern of radioactivity in rats following radiothyroxine injection has been shown to be altered similarly by some but not all antithyroid compounds (4). The data in man and in the rat have been interpreted to indicate that PTU inhibits peripheral deiodination of thyroxine. Inhibition of thyroxine monodeiodinase obtained from rat kiddeiodination of thyroxine. Inhibition of thyroxine deiodination in rat kidney slices following thiouracil and PTU administration (7) have been reported. Methimazole did not appear to affect the excretory pattern of radioactivity in rats (4) nor the thyroxine deiodination by rat kidney slices (7). Slingerland and Burrows (2) found no alteration in radiothyroxine turnover studies in one myxedematous patient given methimazole.

Since methimazole has been used as an experimental tool in the study of peripheral thyroxine metabolism, this study was undertaken to elucidate what effects this compound has peripherally in man.

Material and Methods. The subjects studied included 16 adult volunteers ranging in age from 23 to 62 years. Each was considered to be free of endocrine disease by history and

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[1] Trainee, National Heart Institute, U. S. Public Health Service grant #HTS-5461.