that a HeLa cell scatters as much light as 5 or 6 red blood cells. The latter can therefore be used for checking and adjusting the optical system.

The sensitivity of the control system may be judged from Fig. 11 which shows that a 3% alteration of the incident light is necessary to maintain a constant level of scattered light if the cell density increases from 40 to 50 million/ml. Since the sensitivity of the sensitrol relay is better than 1.5%, it is clear that such an increase of cell density cannot occur without the relay operating and supplying fresh medium. Within this concentration range, the system has a sensitivity to change of about ± 5%.

Summary. The cytostat and its operation have been described. It would seem to be a useful aid to further *in vitro* studies of cell populations.

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Recovery of Reoviruses from Wild and Laboratory Mice. (26948)

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Representatives of the ECHO 10 family, recently designated as the reovirus group(1), have been isolated from man, chimpanzees, monkeys, and cattle, and serologic evidence of infection has been detected in guinea pigs and other mammalian species(1,2). This report describes the isolation of naturally occurring viruses serologically related to reovirus types 2 and 3 from wild mice and laboratory mice, respectively.

Materials and methods. Tissue cultures. Primary trypsinized mouse embryo (ME) and rhesus monkey kidney (MK) tissue cultures were obtained from Microbiological Associates, Inc., Bethesda, Md. Maintenance fluid for ME cultures was 5% horse serum in Eagle's Basal Medium, which was replaced twice weekly. MK cultures were maintained in Mixture 199, without intercurrent fluid change; in later tests, 1% calf serum without gamma globulin (Newborn Agamma calf serum, Hyland Labs., Los Angeles, Calif.) was added. All media contained penicillin (250 u/ml) and streptomycin (250 μg/ml).

Mice. For experimental inoculations, weanling and newborn Swiss mice, strains "General Purpose" and "NIH", were obtained from the Animal Production Section, Nat. Inst. of Health. Mice tested for virus were sacrificed within 24 hours after receipt in the laboratory. Wild mice were live-trapped.

Viruses. As reference viruses, the following reovirus strains were used: type 1, SV

12; type 2, D5; and type 3, Abney. These strains were kindly supplied by Dr. Leon Rosen, who has described their origin(2,3). Standard virus pools for hemagglutination and neutralization tests consisted of supernatant fluids plus cells from infected MK tissue cultures. Pools were stored at 4° or −60°C.

Antiserum. Immune serum was prepared in groups of 20 to 50 weanling mice by giving a single intraperitoneal inoculation of 0.1 or 0.2 ml undiluted MK tissue culture virus, and bleeding 3 weeks later. In some cases a second injection was given at 3 weeks, with bleeding 7 to 10 days later. Individual mouse sera were obtained by orbital puncture or by decapitation, and were prediluted as described previously(4).

Virus isolation. Virus was obtained from suspensions of solid viscera (liver, spleen, kidneys, pancreas, gonads, salivary glands, and brain) or intestines of normal mice, and from transplant-induced tumors. In the course of surveys for mouse polyoma virus, these materials were subjected to one or more of the following virus detection procedures:

1) Tissue culture. 0.05 to 0.1 ml of tissue suspension clarified at 2000 rpm for 15 to 20 minutes was inoculated into 2 to 5 ME or MK tissue cultures, or both. ME cultures were observed for 3 to 6 weeks and passages were made to ME and MK tissue cultures whenever tissue destruction or cellular alteration was noted. With MK tissue culture, blind passages were made routinely at 19 to 21 days, or earlier if definite tissue destruction was noted; cytopathic changes identifiable as those caused by reoviruses were usually not seen in the primary inoculation tubes.

2) Mouse antibody production (MAP) test: Three to 5 weanling mice were inoculated intraperitoneally with 0.1 to 0.2 ml of test material; the mice were bled individually in 28 to 35 days and the sera tested for hemagglutination-inhibiting antibody. Appropriate control mice were included in the test.

3) Newborn mice: One or 2 litters of newborn mice were inoculated with 0.04 ml intraperitoneally; one-half of each litter was sacrificed at 7 days and a suspension of pooled viscera inoculated into a second group of newborn mice, into weanling mice for MAP testing, and into tissue culture. The remaining mice were observed for illness for two weeks.

Hemagglutination (HA) and hemagglutination-inhibition (HI) tests. HA and HI tests were performed in tubes, using the procedure described for polyoma virus(4), or in plates, using the test procedures outlined by Takatsy(5) and Sever(6).

The test system utilized infected MK tissue culture fluid as the source of hemagglutinin, pH 7.2 phosphate-buffered saline as diluent, 1.0% human group O red blood cells, and room temperature incubation. For HI tests, 4 units of hemagglutinin were used and serum-hemagglutinin mixtures were held for 45 minutes at room temperature before addition of erythrocytes; serum was inactivated at 56°C for 30 minutes. For serum surveys, sera were screened at dilutions of 1:40 and 1:80 in tube tests, and 1:20 through 1:80 in plate tests. Antibody titers in hyperimmune mice averaged 1:160 after one inoculation, 1:1280 after 2.

In reovirus HI tests with other species sera, absorption of the serum with kaolin has been used to facilitate reading of tests(2); however, application of this procedure to mouse sera required excessive volumes of serum and resulted in a 4- to 8-fold decrease in homologous antibody; preliminary evaluation of the kaolin procedure as applied to mouse sera did not indicate that the specificity of the test was improved, and therefore kaolin absorption was not used. However, in type 3 reovirus tests using the tube method, sera were absorbed with human group O erythrocytes, since unabsorbed sera gave irregular cell patterns; 1 part of serum diluted 1:10 was mixed with 3 parts 5% erythrocyte suspension and the cells were allowed to settle overnight at 4°C. This procedure was unnecessary when plate tests were employed, since unequivocal readings could be obtained by tipping the plates and observing presence or absence of "tear-drop" slippage patterns.

Neutralization tests. Tissue culture neutralization tests were done in MK tissue cultures, using as the test dose of virus the highest dilution producing at least 3-plus

cytopathic changes in 5 or 6 days (about 10^4 50% tissue culture infective doses). Sera were heated at 56° for 30 minutes and tested at a dilution of 1:10 or 1:20. A serum dilution was considered positive for neutralization if it limited the degree of cytopathic change to less than half that of the virus controls for 24 hours after the controls reached 3-plus.

Results. Isolation of reovirus type 2 from house mice. Mouse embryo tissue cultures inoculated with visceral organ suspensions from 3 wild house mice (#1220, #1223, and #1262), trapped in a single New York City apartment building, developed cytopathic changes consisting of patches and strings of small granular cells appearing after 21 to 26 days in culture. The cytopathic effects spread through the culture very slowly, requiring up to 15 days for complete destruction of the cell sheet. Cytopathic effects were reproduced on subculturing in mouse embryo tissue culture, but with only slightly shortened incubation periods; however, passage to monkey tissue culture resulted within 5 days in the distinctive cytopathic change associated with the reovirus group(7), and Giemsa-stained preparations revealed bluish intracytoplasmic masses of bizarre shape and irregular size identical to those described for the reoviruses (8). The agents were ether resistant; fluids from degenerated monkey kidney cultures agglutinated human group O erythrocytes to an average titer of 1:160; and both mouse embryo and monkey kidney tissue culture passage fluids contained complement-fixing antigen reacting with reovirus type 1 monkey antiserum and with mouse antisera against the reference strains of reovirus types 1, 2, and 3.

Preliminary HI typing of one isolate (#1223) performed in the laboratory of Dr. Leon Rosen with standard guinea pig antisera indicated a relationship to type 2; this was subsequently confirmed by Dr. Rosen in HI tests with rooster antisera to prototype strains, and with paired sera from human and bovine type 2 infections. Simultaneous tests of the latter sera with human, bovine and mouse type 2 HA antigens detected HI antibody rises of nearly identical magnitude. However, in reciprocal HI tests using mouse antisera, the type 2 strains from mice, though sharing antigens with the prototype strain (D5) of reovirus type 2, were antigenically distinguishable from the prototype(9).

Because of the long incubation period required before virus was detected in mouse embryo tissue culture, there was special need for demonstration of infection by additional tests in order to establish the wild mouse rather than the tissue culture assay system as the source of virus. The confirmatory results of reisolation and mouse antibody production tests are given in Table I. It should be noted that preparation of the pooled organ suspensions and inoculation of weanling mice for the MAP test were carried out in a field laboratory with no contact with procedures using reoviruses. Although none of the mice from which virus was isolated had significant serum antibody levels, HI or virus neutralizing activity was detected in the serum of other mice trapped in the virus-positive apartment house, as described below.

A fourth strain of Reo 2 was isolated in monkey kidney tissue culture from intestine suspension of a wild mouse trapped in a barn (Farm W.) in Montgomery Co., Maryland. This strain was serologically identical with the type 2 strains isolated from the New York mice.

Distribution of Reo 2 antibody in mice. Serologic surveys for antibody in wild and laboratory mouse colonies were done by the HI procedure using the #1223 strain as antigen (Table II). Serums from laboratory mice were consistently negative, none of 416 mice from 9 separate colonies demonstrating hemagglutination-inhibition. The occurrence of antibody in the wild mice appeared to be sharply focal in distribution. In the New York apartment house from which the virus-positive mice were trapped, 10% of mice were positive for HI antibody in the first 2 trapping periods; positive mice were found in 4 of the 6 apartment units surveyed, and on 3 of the 5 floors of the apartment house. Although positive mice were widely distributed throughout this apartment house during the first 2 trapping periods, the infection did not appear to persist, since mice trapped 4 to 6 months later from each of the 4 floors were all

TABLE I. Isolation of Type 2 Reovirus from Wild Mice.

Source of isolate	Mouse #	Detection of virus—Tissue culture* ME	MK	MAP test	Reovirus typing HI	Neut	Antibody to reovirus 2 Donors HI	Neut	Contacts† HI	CF
Mus musculus, 128 W. St. 3,‡ N.Y.C.—10% suspension of pooled viscera	1223	+ (25d)	+ (20d)	0/5§	2	2	—	trace		
Idem	1220	+ (26d)	+ (12d)	2/2	2		—	—	8/84	0/5
"	1262	+ (21d)	—	2/5	2		—	—		
Mus musculus, barn, W. farm, Montgomery Co., Maryland —10% intestine suspension	D338		+ (7d)‖	0/3	2		—		5/75	0/3

* Numbers in parentheses are the day of first appearance of cytopathic effects.
† Mice trapped in same location during comparable time period.
‡ Street number is coded(10).
§ Fractions = No. positive/No. tested.
‖ Atypical cytopathic effects seen at 7 days.

negative for antibody. Mice trapped in 11 other apartment houses on the same block during the period when positive mice were found were all negative for antibody.

A similar focal distribution was found in the farm mice. The highest incidence of antibody was in the W. farm from which the virus-positive mouse was recovered; 4 other colonies of rural wild mice yielded 1 positive animal each.

The HI antibody titers in the wild mice were as follows: 1:20 in 4 mice; 1:40 in 7; 1:80 in 1; and greater than 1:80 in 5. None of 10 of the positive wild mouse sera gave a significant reaction when tested at a dilution of 1:10 in complement fixation test against type 2 antigen.

Isolation of Reovirus type 3 from laboratory mice. Following the detection of type 2 reovirus infection in wild mice, additional strains of reovirus were obtained from laboratory mice. All of the 7 strains obtained to date have been identified as type 3 (Table III). Two of the strains were obtained from organ suspensions of normal mice; 4 from ascites-form transplanted mouse leukemias (6 were tested), and one from supernatant fluids from explant cultures of leukemic tissues of a mouse bearing a transplant of Moloney virus-induced leukemia. The isolates produced the characteristic reovirus cytopathic effects in mouse embryo and monkey kidney tissue cultures with typical cytoplasmic inclusion bodies, and when inoculated into newborn

TABLE II. HI Antibody to Reovirus 2 in Mice.

Area	Period of serum collection	No. mice positive/No. tested, by colony	Total
Wild mice—New York City, 128 W. St. 3	Aug. - Oct., 1959	4/43*	4/43
Idem	Jan., 1960	4/41	4/41
"	June - Aug., 1960	0/99	0/99
Other buildings, W. St. 3	Aug., 1959 - June, 1960	0/114, 0/20, 0/13, 0/9, 0/9, 0/7, 0/6, 0/5, 0/4, 0/3, 0/2	0/192
Wild mice—Maryland Farms	Aug., 1960 - June, 1961	5/76,* 1/68, 1/25, 0/56, 0/26, 0/7	7/258
—Grain Mills	*Idem*	1/107, 1/37, 0/45, 0/18	2/207
Laboratory mouse colonies	1959 - 1961	0/140, 0/51, 0/50, 0/39, 0/33, 0/30, 0/27, 0/23, 0/23	0/416

* Areas and periods in which virus-positive mice were trapped.

TABLE III. Isolation of Type 3 Reovirus from Laboratory Mice.

Source of isolate	Detection of virus: Tissue culture ME	Tissue culture MK	MAP test	Suckling mice*	Reovirus typing HI	Reovirus typing Neut	Serum inhibitor of reovirus 3 in donor mice HI	Serum inhibitor of reovirus 3 in donor mice Neut
DBA/P retired breeders, NIH colony, 10% suspension of viscera of 12 mice.	+†		1/3	+	3	3	3/12	4/12
RFM retired breeders, Oak Ridge Nat. Lab. colony, 10% suspension of viscera of 9 mice.	—		0/2	+	3	3	7/10	7/9
Supernatant fluid from explant cultures of leukemic lymph node tissue from mouse with transplanted Moloney-virus leukemia.	+ (17d)				3	3		
Transplanted mouse leukemia, 195/8‡		+ (13d)	5/5	(+)	3			
Transplanted mouse leukemia, L1210/6MP/FU/AM‡		+ (13d)	5/5	(+)	3			
Transplanted mouse leukemia, P388/8‡		+ (13d)	5/5	(+)	3			
Transplanted mouse leukemia, 815/FU‡		+ (10d)	5/5	(+)		3		

* + = Virus recovered from tissues of suckling mice inoculated with test suspensions.
(+) = Suckling mice inoculated with test material developed characteristic picture of reovirus infection; no attempt made to recover virus from their tissues.

† Virus isolated by blind passage at 30 days.

‡ Dr. L. W. Law, suspension of tumor in ascites fluid.

mice induced the characteristic oily hair disease described for reovirus infection(3,11). Also, the tissue culture fluids infected with these isolates showed the characteristically low titers of hemagglutinin observed with type 3 strains recovered from human sources.

Reciprocal hemagglutination - inhibition tests with mouse antisera against 3 of the mouse isolates and the prototype Abney strain indicated only minor differences in antigenic composition between the mouse and human isolates.

Attempts have been made to determine the prevalence and distribution of reovirus type 3 infection in laboratory and wild mouse populations using hemagglutination inhibition and neutralization test procedures. However, serum inhibitors of both hemagglutination and infectivity were so highly prevalent among retired breeder laboratory mice, including mice of specific-pathogen-free and, in some instances, germ-free colonies, that the possibility of non-specific serum inhibitors must be considered, particularly since the titers encountered were generally low (1:20 to 1:80). Attempts to evaluate the significance of the serum inhibitors by correlative complement fixation and virus isolation studies are in progress. Serum inhibitor of type 3 hemagglutination and infectivity has also been found in wild mice, but less frequently than in the majority of laboratory mouse colonies.

Discussion. The serologic survey data presented here suggest that type 2 reovirus infection may not be an indigenous infection of the mouse, but that the infections observed may have been acquired from contact with other species. This was suggested by the low frequency of antibody in the positive foci and by the apparent disappearance of infection from the New York focus. If this supposition is correct, it would appear likely that the New York mice acquired the infections from humans and that the Maryland farm mice may well have acquired the infection from cows. However, it is perhaps significant that

the strains isolated from the 2 different areas were of the same subtype. The possibility must be considered that the infected mice could serve as a source of infection for humans and other animals.

Although the question was raised that the serum inhibition of type 3 reovirus may be due to non-specific inhibitors, the type 2 serologic results would appear to be specific, since the occurrence of antibody correlated well with the presence of mice infected with the virus. The possibility that some of the type 2 reactions may represent cross reactions from infection with other reovirus types must be considered; however, no correlation has been observed between the presence of type 3 inhibitor and inhibition of type 2 in HI tests of individual mouse sera, and tests with sera of immunized mice indicate that the HI and neutralization procedures give only minimal cross reaction.

The finding of reovirus type 3 virus in 5 of 7 transplanted mouse leukemias suggests that this virus may be a relatively frequent contaminant of mouse tumors. Also, we have identified the oncolytic virus isolated by Nelson(12) from a transplantable ascites tumor as a strain of reovirus type 3.

Summary. Using virus isolation and serologic survey procedures, the occurrence of natural reovirus infection in mice has been detected. Four isolates of reovirus type 2 were recovered from urban and rural wild mice; 7 strains of reovirus type 3 were isolated from normal laboratory mice and transplant-induced mouse leukemias. Antibody to type 2 was found focally distributed in wild mice, but was not encountered in laboratory mice. A low titer serum inhibitor of type 3 HA and infectivity, possibly representing specific antibody, was prevalent in laboratory mice.

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Effects of Gonadotrophins and Androgens on the Sex Organs of the Estrogen-Treated Male Rat. (26949)

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In an effort to use estrogen-treated immature male rats for LH assays it became apparent that the ventral prostate response to crude and purified preparations was quite different. Data obtained in examining these differences are here presented.

Methods. In the first series of experiments, 17-18 day old male rats (Charles River Lab.) were given a single subcutaneous injection of 5 μg of estradiol dipropionate in peanut oil. In the second series, 20-21-day-old Sprague Dawley rats were injected with 10 μg of estradiol dipropionate in peanut oil. The animals were fed *ad libitum* with Purina Lab Chow.

Subcutaneous injections of the materials