

TABLE I. Effect of Hyperbaric Oxygen on Maternal Mortality, Fetal Resorption, and Gross Congenital Malformations in Golden Hamster.

Day of gestation treated	Mothers treated (No.)	Mothers surviving (No.)	Fetuses surviving (No.)	Fetuses resorbed (No.)	Fetal resorption rate (%)	Fetuses malformed (No.)
8*	5	5	60	2	3	0
8†	15	13	139	20	12	3
8‡	5	4	42	13	24	2
7*	6	6	66	2	3	0
7†	6	5	63	3	5	2
7‡	8	5	62	4	6	9
6†	4	4	44	3	6	1
6‡	4	2	26	0	0	0

* 30 lb/in² for 3 hr.† 40 lb/in² for 3 hr.‡ 45 lb/in² for 2 hr.

proaches the maximal maternal stress since about one-fourth of the mothers succumbed to this insult. None of the malformations noted here have been found in any of the animals run at 30 lb/in² for 3 hours or in any offspring of over 300 hamster pregnancies used as controls in other teratogenic experiments in this laboratory. Repeated attempts by this author to produce experimental malformations in the golden hamster by reduced atmospheric pressure equivalent to 18,000 ft elevation for 12 hours have been unsuccessful. With the increasing use of hyperbaric oxygen therapy in medical practice, attempts should be made to study this teratogenic effect in other species and to determine the relative roles of increased oxygen tension and increased atmospheric pressure in embryonic development.

Summary. Pregnant golden hamsters in early stages of gestation were exposed to hyperbaric oxygen at 3.0, 3.6, and 4.0 atmospheres for periods of 3 and 2 hours. One-

fourth of the mothers developed central nervous system signs during treatment and were sacrificed. A small but significant number of fetuses recovered late in gestation had severe gross congenital malformations, consisting of umbilical hernias, exencephaly, spina bifida, and limb defects.

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Additional Rhinovirus Serotypes. (29426)

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The rhinoviruses(1) are presently believed to be a principal cause for common cold in adults and a frequent cause for certain upper and lower respiratory tract illnesses in chil-

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dren. The rhinovirus group includes all agents known previously as JH-2060(2), ECHO 28 (2), coryzavirus(3,4), rhinovirus(5), murivirus(6), ERC agents (ECHO 28-rhinovirus-coryzavirus group)(4), and enterovirus-like viruses(7). Family relationship is based on

the common properties of small size, RNA type, ether stability, and extreme lability of infectivity at pH 3.0(1,4,5,8). Certain of the viruses of the group propagate only in cells of human origin (H strains) while others propagate both in human and monkey cells (M strains)(9).

In a previous report from this laboratory (4), 37 rhinoviruses were described and these were divided into 20 distinct serotypes. Thirteen different rhinoviruses were described by others(2,7,9-12). The present report describes the isolation of 56 additional rhinovirus strains, 29 of which belong to 20 new serotypes. This brings the total of defined rhinovirus types to 53. Data relating to serologic responses to infection in man and to immunologic homogeneity within types are also presented.

Materials and methods. Clinical specimens. The clinical populations consisted of employees of Merck and Co., Rahway, N. J.; students at University of Pennsylvania; and children seen in the outpatient clinics or on the wards of the Children's Hospital of Philadelphia. The patients were selected on the basis of having an acute respiratory illness of 4 days or less duration judged on clinical grounds to be of viral etiology. Persons without evident respiratory illness were included for control purpose. All patients were entered in the study during the period from September 1960 through June 1962. Throat samples and a blood specimen were collected from each patient at the time of initial visit and a second blood sample was taken approximately 3 weeks later. The methods employed for collecting and handling the materials were the same as described earlier(3,4). *Cell cultures.* Cultures of the WI-26 strain of diploid human fibroblasts(13) were propagated in Eagle's minimal essential medium (EMEM) in Earle's salt solution containing 10% agamma calf serum and 0.15% sodium bicarbonate. The maintenance medium for the WI-26 strain of cells was EMEM containing 2% inactivated horse serum, 0.18% sodium bicarbonate, and 0.006M tris (hydroxymethyl aminomethane) buffer. Trypsinized grivet renal cells were planted in Hanks' balanced salt solution containing 0.5% lactalsate,

0.25% yeast extract and 2% agamma calf serum. Four to 6 days after planting, the cells were refed with Medium 199 containing 2% calf serum. The grivet cells were maintained in EMEM containing 2% inactivated chick serum and 0.33% sodium bicarbonate. HeLa cell cultures were grown in Eagle's basal medium in Earle's salt solution (EBME) with 5% agamma calf serum and were maintained in EMEM containing 5% agamma calf serum and 0.15% to 0.30% sodium bicarbonate depending upon the cell population at time of virus inoculation. All media contained 100 units of penicillin and 100 μ g of streptomycin per ml, and additionally, maintenance media contained 100 μ g of neomycin per ml. Roller tube cultures were maintained with 1.8 ml of medium and the dose of virus inoculum was 0.2 ml. Inoculated tube cultures were incubated rolling at 33°C unless otherwise specified. *Virus isolation.* The technique for virus recovery from throat specimens was described earlier (3,4). In the present study, all strains were isolated in single passage in the WI-26 cells and all isolates were adapted to growth in HeLa cell culture. *Prototype strains.* Table I presents a summary list of strains of rhinovirus included in the present study and indicates their source and published literature reference. Additionally, strains NIH 353 and 179E were furnished by Dr. K. M. Johnson and Dr. D. Hamre, respectively. None of the strains has been "purified" by picking plaques or by terminal dilution passage, as for all viruses other than enteroviruses, such purported purification is not a condition for classification as a rhinovirus(1). *Antisera.* Reference antisera against strains 106F, 140F and 179E were kindly supplied by Dr. M. Mufson. All other antisera were prepared as described earlier(4). *Serum neutralization tests.* These tests were performed as described previously(4) using HeLa cell cultures. Between 100 and 1000 TCD₅₀ of virus were employed in tests with guinea pig antisera and between 10 and 100 TCD₅₀ in tests with human sera. *Serologic typing of isolates.* Initial typing was carried out by the "intersecting serum scheme" neutralization technique utilizing serum pools according to

TABLE I. Prototype Reference Strains Used.

SEROTYPE	PROTOTYPE STRAIN	LITERATURE REFERENCE	OBTAINED FROM
1	2060	1	DR. A. SABIN
2	SHEFF./1/60H (1131)	9	DR. D. TAYLOR-ROBINSON
3	SAL./1/59H (1134)	"	" "
4	SAL./1/56H (FEB)	"	" "
5	SAL./1/51H (1045)	"	" "
6	SAL./1/60M (1236)	"	" "
7	SAL./1/57H (HGP)	"	" "
8	NIH (1734)	7	DR. K. M. JOHNSON
9	NIH (11757)	"	" "
10	NIH (33342)	"	" "
11	68	4	THIS LABORATORY
12	MRH	"	" "
13	211	"	" "
14	204	"	" "
15	1	"	" "
16	181	"	" "
17	5682	"	" "
18	93	"	" "
19	15	"	" "
20	21	"	" "
21	47	"	" "
22	127	"	" "
23	5007	"	" "
24	5124	"	" "
25	5146	"	" "
26	5426	"	" "
27	5660	"	" "
28	5670	"	" "
29	6101	"	" "
30	5582M	"	" "
31	NIH (1059)	7	DR. K. M. JOHNSON
32	106F	12	DR. D. HAMRE
33	140F	"	" "
34-53*	NEW VIRUSES TO BE DESCRIBED (20 TYPES)		THIS LABORATORY

* PROTOTYPES OF NEW VIRUS TYPES 34-53 ARE DESIGNATED IN TABLE II.

Schmidt *et al*(14). Polyvalent pools of guinea pig antisera containing 20 units of antibody against each type of virus were employed. Typing accomplished by this technique was confirmed in tests with the corresponding monovalent antiserum. The serologic identity of each new isolate with those of previous types was established in appropriate tests employing paired sera from the human being from whom the virus was recovered and the corresponding prototype virus strain. Cross-neutralization tests designed to measure antigenic homogeneity within types were carried out using the viruses under study and their corresponding guinea pig antisera. *Differentiation of H and M strains.* Each new rhinovirus isolate was identified as to H (human) or M (monkey) subgroup. For this purpose, HeLa cell grown virus with high infectivity titer was passed

undiluted to 4 grivet renal cell cultures which were incubated at 33°C in the roller drum. The cultures were examined every 2 or 3 days for cytopathology for a period of 10 to 13 days. One blind passage was made for each strain which failed to show a cytopathic effect on first passage. A known positive M strain 5582M, was included in all tests for control purpose. Viruses which caused cytopathology in grivet renal cells were designated M strains and those which failed to do so were called H strains. The specificity of the cytopathology caused by each M strain was established in appropriate serologic identity tests using the grivet cell passage virus as seed. *Sedimentation studies.* Tests to establish small size of the rhinoviruses were carried out as described earlier(4) using adenovirus 5 grown under identical conditions for comparative purpose. All viruses tested were propagated in HeLa cell culture and centrifugation was for 15 minutes at an average centrifugal force of $108,786 \times g$. *Test for lability at pH 3.0.* These tests were carried out by a previously described method (4). The pH of the test and control samples at the beginning and end of the test period ranged from 2.9 to 3.1 and from 6.9 to 7.0, respectively. Viruses stable at pH 3.0 were included as controls. All infectivity titrations were performed using HeLa cell cultures. *Nucleic acid determination.* Nucleic acid typing was according to Salzman(15) and as described earlier(3,4,16) with the exception that BUDR (5-bromo-2'-deoxyuridine) was substituted for the 5-fluoro derivative, FUDR. Vaccinia, a known DNA virus, and rhinovirus type 11 strain 68, a known RNA virus, served as controls. *Essential lipid.* Tests for essential lipid were performed according to Feldman and Wang(17) using chloroform. All titrations for infectivity were done in HeLa cell cultures employing 8 tubes per virus dilution. Respiratory syncytial virus was included as the chloroform-sensitive virus control.

Results. Recovery of rhinovirus strains. As seen in Table II, 56 rhinovirus strains were recovered. Fifty-two of the strains were derived from 672 cases of respiratory disease in adults or in children. Four of the strains

TABLE II. Essential Information Relating to 56 New Rhinovirus Strains.

Rhinovirus		Patient		Diagnostic neutralization test	
Type	Strain	Age (yr)	Diagnosis	Acute	Convalescent
4	1005	19	URI	Not done	Not done
6	6211M	1	Asthmatic bronchitis	< 5	160
8	6167	16 wks.	Control	< 5	160 or >
"	6268	3	Bronchopneumonia	< 5	40
"	1098	21	URI	< 2.5	10
9	6367	1 1/2	URI	< 5	80
"	6698	6	Control	< 2.5	10
"	1121	20	URI	< 2.5	5
12	237	48	URI	< 2.5	20
"	1016	18	URI	< 5	20
"	1106	18	URI	< 2.5	40
"	1168	18	URI	< 5	80
"	1209	21	URI	< 5	160 or >
"	1326	21	Bronchitis	< 2.5	5
"	1444	18	URI	< 5	160 or >
20	225	50	URI	< 2.5	20
22	1924	21	URI	< 5	40
"	6554	4	URI	< 2.5	40
27	6166	2	Control	< 5	40
32	1482M	18	URI	< 2.5	80
"	1539M	18	Control	< 2.5	160 or >
"	1955M	21	URI	< 2.5	20
"	6498M	3	Asthmatic bronchitis	< 2.5	160 or >
"	6503M	8 wks.	Bronchiolitis	< 2.5	20
"	6526M	1	Asthmatic bronchitis	< 2.5	160 or >
33	465M	34	URI	< 2.5	40
"	1665M	22	URI	< 2.5	20
34	515*	34	URI	< 2.5	10
35	611*	48	URI	< 2.5	20
36	1770*	19	URI	< 2.5	< 5
37	2268*	19	URI	< 2.5	640
38	6660*	3	Bronchitis	< 2.5	10
39	6669*	1	Bronchiolitis	< 2.5	160
40	1963M*	20	URI	< 2.5	40
"	2219M	21	URI	< 2.5	10
41	6360*	6 wks.	Bronchiolitis	20	160
"	6244	1 1/2	Bronchopneumonia	< 2.5	40
42	6692*	1	URI	< 2.5	40
"	457	39	URI	< 2.5	40
"	474	38	URI	< 2.5	80
43	1936*	20	URI	< 2.5	5 (P)**
"	463	42	URI	< 2.5	20
"	1970	22	URI	< 2.5	10
44	6258*	8 wks.	URI	< 2.5	80
"	1863	23	URI	< 2.5	10
"	1958	16	URI	< 2.5	10
45	605*	58	URI	< 2.5	20
46	1979M*	19	URI	< 2.5	20
47	425*	20	URI	< 2.5	20
48	1983*	18	URI	< 2.5	80
49	2253*	29	URI	< 2.5	5
50	477*	42	URI	< 2.5	5 (P)**
51	1857*	25	URI	< 2.5	20
52	1671*	20	URI	< 2.5	20
"	1938	19	URI	< 2.5	5
53	464*	59	URI	< 2.5	5

* New viruses designated as prototype strains.

** Partial but incomplete neutralization at a serum dilution of 1:5.

were from 204 of the control cases examined. Forty-two of the cases from which virus was recovered were upper respiratory illness and 10 were lower respiratory illness diagnosed clinically as bronchitis, asthmatic bronchitis, bronchiolitis, or bronchopneumonia. All but one of the cases with lower respiratory tract involvement were in children less than 4 years of age. The one adult, case 1326, was 21 years of age and presented as bronchitis.

All but one (1770) of the 51 respiratory disease cases for which paired sera were available showed a significant increase in homologous antibody level during convalescence. The convalescent serum titers ranged from 1:5 to 1:640. In 2 instances (1936 and 477), neutralization was not complete. All 4 persons in the control group from whom virus was recovered did develop homologous neutralizing antibody, even though they were asymptomatic.

Serologic typing of strains. Extensive serum neutralization tests were carried out to compare the newly isolated rhinoviruses with those described earlier by our group(3, 4) or by others(2,7,9-12). Strain 353 of Johnson and Rosen(7) was found to belong to rhinovirus type 23 and strain 179E of Connelly and Hamre(12) was a type 30 virus. Both these types were described earlier by us(4). Among the 56 new isolates described here (Table II) 27 were similar to previously described agents. One (1005) of the 27 strains belonged to type 4 and another (6211M) to type 6 of Taylor-Robinson *et al*(9). Three strains (6167, 6268, 1098) belonged to type 8 and three (6367, 6698, 1121) to type 9 of Johnson and Rosen (7). Six strains (1482M, 1539M, 1955M, 6498M, 6503M, 6526M) belonged to type 32 and two strains (465M, 1665M) to type 33 of Connelly and Hamre(12). Seven strains (237, 1016, 1106, 1168, 1209, 1326, 1444) belonged to type 12, one (225) to type 20, two (1924, 6554) to type 22 and one (6166) to type 27 previously described by us(4). Twenty-nine of the new isolates belonged to 20 new serotypes which are now established and which number from types 34 through 53. A total of 30 serotypes were represented among the 56 newly recovered rhinovirus

strains. Types 12 and 32 were encountered most frequently with 7 and 6 strains each and there were 5 types with 3 strains each and 5 types with 2 strains each. Eighteen types were represented by a single strain each.

Biological, biochemical and biophysical characterization of new rhinovirus isolates.
Cytopathology. All the new isolates caused typical cytopathology in WI-26 cells(3) characterized by separation of the cells in the sheet, development by individual cells of a rounded, oval or angular form with refractile property and eventual lysis. Characteristic cell rounding and swelling followed by shrinking and granularity were seen in HeLa cell culture. All the isolates were tested in cultures of primary rabbit kidney and none were found to be cytopathic.
M and H subgrouping. Thirty-three selected strains, including all prototypes, were tested in renal cells of grivet monkey. As shown in Table II, nine of the isolates were of M subgroup and belonged to previously described types 6, 32 and 33. Among the new rhinoviruses, strains of types 40 and 46 were of M type. All other isolates were H strains.
Lability at pH 3.0. All the new isolates were tested for lability at pH 3.0 and all were labile. The infectivity titers of the pH 7.0 control samples ranged from $10^{3.0}$ to $10^{4.5}/0.2$ ml. At pH 3.0, infectivity titers were less than $10^{1.0}/0.2$ ml.
Nucleic acid typing. One strain of each type represented in Table II was tested for nucleic acid type. In the case of our new types 34-53, the particular strains designated as prototypes were so tested. None of these viruses were inhibited by BUDR.
Essential lipid. The same strains which were typed for nucleic acid composition were tested for sensitivity to chloroform. There was no significant difference in titer between treated and untreated viruses and all the strains were judged not to contain essential lipid.
Sedimentation studies. Viruses subjected to sedimentation studies were type 32 strain 1482M, type 41 strain 6360, type 42 strain 6692, type 44 strain 6258, type 46 strain 1979M and type 52 strain 1671. None of these strains showed a significant reduction of infectivity titer in the supernate compared to

TABLE III. Intratypic Serological Relationships Between Rhinovirus Strains Employing Guinea Pig Antisera.

RHINOVIRUS GUINEA PIG ANTI-SERUM		RECIPROCAL OF NEUTRALIZING ANTIBODY TITER OBTAINED																							
		TYPE 32						33	40	41	42		43		44		52								
T Y P E	S T R A I N	1 4 8 2 M	1 5 3 9 M	1 9 5 5 M	6 4 9 8 M	6 5 0 3 M	6 5 2 6 M	4 6 5 M	1 6 6 5 M	1 9 6 3 M	2 2 1 9 M	6 3 6 0	6 2 4 4	6 6 9 2	4 5 7	4 7 4	1 9 3 6	4 6 3	1 9 7 0	6 2 5 8	1 8 6 3	1 9 5 8	1 6 7 1	1 9 3 8	
32	1482M	640	640	640	640	640	640																		
"	1539M	640	640	640	640	640	640																		
"	1955M	640	640	640	640	640	640																		
"	6498M	640	640	640	640	640	640																		
"	6503M	320	320	320	320	640	640																		
"	6526M	640	640	640	640	640	320																		
33	465M							640	640																
"	1665M							80	160																
40	1963M									160	320														
"	2219M									320	320														
41	6360											320	160												
"	6244											640	640												
42	6692													640	640	640									
"	457													320	640	640									
"	474													640	640	640									
43	1936																ND*	ND	ND						
"	463																640	640	640						
"	1970																640	640	640						
44	6258																			320	320	320			
"	1863																			640	640	640			
"	1958																			320	640	320			
52	1671																						320	320	
"	1938																						320	640	

1: 640 WAS THE HIGHEST SERUM DILUTION TESTED.

* NOT DONE.

the control sample. The adenovirus control, in each instance, showed a reduction in titer on centrifugation of 2.75 logs or greater. This established the small size of the rhinovirus strains examined.

Serologic homogeneity within rhinovirus serotypes. As shown in Table II, several of the new serotypes were represented by more than one strain. All new strains belonging to types 32, 33, 40, 41, 42, 43, 44 and 52 were crossed in serum neutralization tests to detect possible antigenic heterogeneity within these types. The findings shown in Table III reveal a remarkable degree of homogeneity within types, which is like that displayed by enteroviruses and is unlike that shown, e.g., by arboviruses, influenza A and B viruses and others. A slight reciprocal antigenic relationship, not shown in the table, was found between types 52 and 53 strains.

Confirmation of serotyping using paired sera from human cases of rhinovirus infection. Our previous findings(4) revealed a remarkable degree of specificity in the serologic response of man to a particular virus type. Paired sera from human cases of rhinovirus

infection, therefore, have value for preliminary typing of new isolates or in confirming serotype identifications made in tests with animal antisera. Table IV presents the serologic findings obtained in studies in which paired sera from various cases of rhinovirus infection in the present study were tested with the homologous virus isolate and with the corresponding prototype strain. Total agreement in the typing obtained with animal antisera was established and further, a reciprocal serologic identity of the strains with the prototype virus was shown.

Discussion. The findings present continuing evidence for the important role of rhinoviruses in acute respiratory illnesses among children as well as among adults. The proportion of cases of total acute respiratory disease caused by rhinoviruses cannot be determined at this time since there is no basis for assessing the efficiency of the virus isolation procedure for diagnostic purpose. In the total group, only 52 (8%) of 672 cases of respiratory illness yielded a rhinovirus. This was the same order of magnitude reported in earlier studies(18,19) from our laboratory.

TABLE IV. Confirmation of Rhinovirus Serotypes in Tests with Paired Sera from Human Rhinovirus Infections.

PAIRED HUMAN SERA			NEUTRALIZATION ANTIBODY TITERS OBTAINED IN TESTS WITH VIRUS TYPE AND STRAIN																								
RHINOVIRUS TYPE	CASE & STRAIN NO.	SERUM SAMPLE	TYPE 6		8		9		12						20		22		27								
			6211M	1236M	6167	6268	1098	1734	6367	6698	1121	11757	237	1016	1106	1168	1209	1326	1444	MRH	225	21	1924	6554	127	6166	5660
6	6211M	ACUTE CONVAL.	<5	<5																							
8	6167	A																									
"	6268	A																									
"	1098	A																									
9	6367	A																									
"	6698	A																									
"	1121	A																									
12	237	A																									
"	1016	A																									
"	1106	A																									
"	1168	A																									
"	1209	A																									
"	1326	A																									
"	1444	A																									
20	225	A																									
22	1924	A																									
"	6554	A																									
27	6166	A																									

However, in view of the inability, to date, to define the etiology of a sizeable proportion of respiratory disease cases, the possibility must be entertained for existence of other kinds of viral agents in respiratory disease which are not as yet discovered.

All but one of the rhinovirus isolations from adults were from cases of common colds (upper respiratory illness, URI). This one exception was a case of bronchitis. By contrast, 9 of the 13 isolations in children were from lower respiratory tract illness and these were diagnosed as bronchitis, asthmatic bronchitis, bronchiolitis or bronchopneumonia. Such frequent association of rhinoviruses in lower respiratory tract illnesses in children (4,19) and the infrequent occurrence in adults is in agreement with earlier findings (4,19-22). Details relating to the epidemiologic aspects of the present study will be reported later.

We previously (4) described 20 rhinovirus serotypes which were designated types 11 through 30. Twenty new serotypes were recognized among the 56 strains of virus recovered in the present studies. These were designated types 34 through 53. Additionally, 13 serotypes were recognized among strains of rhinovirus recovered by other workers and these were designated types 1 through 10, and 31 through 33. Insofar as was possible, assignment of type number was sequential, based on the time when the viruses were reported in the literature. Additional strains of virus have been recorded (6) which probably belong to the rhinovirus group, but their properties have not been defined and they have not been studied in this laboratory.

The rate and extent of progress which can be made in any field must necessarily be related to the simplicity or complexity of its

technology. The working methods for studies of rhinoviruses have now been so simplified as to make rapid progress quite possible in the ordinary laboratory(16). The methodology for investigations of these agents was greatly improved by application of the human diploid fibroblast cell culture for virus isolation and propagation purposes(3,13). The demonstration of acid lability of rhinoviruses(4,8) has provided an easy and practicable method for differentiating them from enteroviruses and has eliminated the need for laborious cross-testing of all new rhinovirus isolates against the numerous serotypes of enterovirus. Tests for nucleic acid type using BUDR and for essential lipid employing chloroform, which are necessary for establishment of new strains as rhinoviruses, are now easy of achievement in the routine sense. Determination of virus particle size is still a laborious procedure but is not an essential property which must be measured for practicable rhinovirus identification purpose(16). Finally, demonstration of typical rhinovirus cytopathic changes in human diploid and in HeLa cell cultures is an invaluable aid in rhinovirus identification.

Progress toward definition of etiology in any viral disease poses the invariable question of what to do about its control from the immunologic or vaccine aspect. Various studies(3,4,12,23-27) have shown that recovery from infection is accompanied by appearance of homotypic antibody in titers as great as 1:640, thus providing a basis of hope for the immunologic approach. Additionally, such antibody is protective(23,24,26,27). The principal problem, from the standpoint of contemporary knowledge, is the probable very large total number of different serotypes and the extreme specificity of homologous serologic response to infection, even among adults who have presumably had a very broad prior experience with agents of this group. Such diversity of serotypes gives ready explanation to the mystery of the recurring common cold but provides little assurance for development of a practicable and efficacious vaccine. Vaccine development must rest either on new principles not readily apparent at this time or on factual informa-

tion, which is not presently available.

Summary. Fifty-two rhinovirus strains were isolated from 672 cases of acute respiratory illness in children and adults and 4 strains were recovered from well controls. Respiratory disease cases among adults which yielded a rhinovirus were nearly all upper respiratory illness (common cold) whereas the cases among children often showed lower respiratory tract involvement. Nearly all cases revealed a homotypic neutralizing antibody response during convalescence. Among the total 56 strains isolated, 20 new serotypes were found. These 20 new types added to the 20 types described previously by our laboratory and 13 types recognized by other workers bring the total number of known serotypes to 53. The extreme diversity of serotypes among rhinoviruses goes far toward explaining the recurrent nature of the common cold and emphasizes the likely difficulty for development of effective vaccines for protection against this syndrome.

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Effect of Magnesium on Uptake and Retention of Radioactive Strontium.* (29427)

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It has been reported that the feeding of magnesium ions increased the elimination of radioactive strontium by rats that had received the isotope either one or 30 days before treatment(1). Magnesium ion is a potent hypercalciuric agent(2,3) and it was presumed that the increased Sr^{89} elimination via the kidney accompanied the enhanced calcium excretion. This effect of magnesium was evident as late as 30 days after administration of the tracer; similar results have been obtained when Ca^{45} was used as a tracer(4,5). To date, no procedure based on a carrier effect(6) acidosis(7) or chelation(8) has had any effect on the turnover of strontium that had been deposited in bone for any length of time before initiation of treatment. A low phosphorus diet has been

shown to decrease uptake of Sr^{89} by the skeleton(9,10) and more recently it was observed that a low-phosphorus diet initiated 3 months after injection of Sr^{90} - Y^{90} into mice did decrease its retention(11). The following studies were designed to investigate further and quantify the effects of Mg^{++} on the removal of "bone-fixed" strontium, since any procedure which could enhance the turnover of skeletal strontium would be of practical importance in treatment of the internal radiation hazard from Sr^{90} . Use of a gamma emitter, Sr^{85} and specialized whole body counting apparatus made possible the direct and repeated *in vivo* measurements of Sr^{85} retention as a function of time following treatment.

Experimental. Rats of the Sprague-Dawley strain were used. In Part I, the groups, labeled young adult rats, had an average weight of 230 g and the groups labeled old adult rats (Groups 8 and 9) weighed ap-

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