chlorothiazide both sodium reabsorption and O_2 consumption are lowered. Urine volume and C_{osm} are elevated, U/P_{osm} and U/P_{Creat} decreased while $T^c_{H_2O}$ remains unchanged.

Plotting Na reabsorption (μeq/g min) against O_2 consumption (μmol/g min) results in a straight line which intercepts the O_2 consumption axis at 1 μmol/g min. We believe that this value may be equated with renal basal O_2 consumption; *i.e.*, without Na reabsorption. The O_2 requirement for renal sodium reabsorption is calculated to be 28.6–32 μeq Na/μmol O_2.

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Studies of Acute Respiratory Illnesses Caused by Respiratory Syncytial Virus. 1. Laboratory Findings in 109 Cases. (26452)

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Morris *et al.*(1), described the first recovery of a virus, termed chimpanzee coryza agent (CCA), from the respiratory tract of chimpanzees with an acute respiratory illness and from a laboratory worker who had been in contact with these animals. The infected chimpanzees and the laboratory worker developed complement fixing and neutralizing antibodies in response to their infections. Chanock *et al.*(2,3) reported recovery of a similar virus from 2 infants with lower respiratory tract infection and showed antibody titer increases among persons with respiratory illness as well as controls; these workers were not able, however, to provide definitive evidence for etiologic association between the virus and human illness. Chanock renamed this agent the respiratory syncytial virus. Since that time, Rowe *et al.*(4) have reported positive laboratory diagnostic findings for the respiratory syncytial agent in an infant with pneumonia. More recently, Beem *et al.*(5) have recorded laboratory findings in 41 cases of respiratory illness among children infected with the respiratory syncytial agent.

Since 1959, our clinical-laboratory groups have engaged in extensive investigations of the etiology and epidemiology of acute respiratory illness among patients admitted to the wards or to the outpatient clinics of the Children's Hospital of Philadelphia or the Hospital of the University of Pennsylvania. The present report describes the laboratory findings among 109 cases of acute respiratory illness shown to be caused by the respiratory syncytial virus. Subsequent reports(6,7) in this series describe the epidemiology of infections with the virus and the spectrum of clinical findings among patients with illnesses caused by this agent.

Materials and methods. Clinical population. The patients were children less than 8 years of age seen in outpatient clinics or admitted to wards of the Children's Hospital of Philadelphia or the Hospital of the University of Pennsylvania, and selected on the basis of having an acute respiratory illness of 4 days or less duration judged on clinical grounds to be of probable viral etiology. The majority of persons were from a low socioeconomic group, mostly Negro, and scattered geographically over a large area of Philadelphia. The cases described occurred during the period from Oct. 1959 through June

1960. Altogether, 563 cases of respiratory illness were studied. *Collection of specimens.* When seen initially, each child was examined clinically and 2 dry cotton swab samples were collected from the throat. These were extracted immediately into 5 ml of Difco veal infusion broth in a screw-cap tube, frozen in dry ice and transmitted to the laboratory where they were thawed, distributed and stored frozen in dry ice in flame-sealed glass containers until tested up to 1 year later. A 12 ml sample of blood was taken at the first visit and, routinely, 21 days later although there was delay in some instances owing to failure of the patients to return at the designated time. The sera were stored frozen at −20°C until tested up to 1 year later. Routine bacterial cultures of the throat failed to reveal primary bacterial etiology in any of the cases selected for study.

Laboratory. Tissue cultures. HeLa cell cultures were grown in Eagle's basal medium (8) with 10% inactivated normal horse serum and the WISH continuous cell line of human amnion(9) was propagated in Eagle's medium containing 10% inactivated normal calf serum. During virus propagation, all cell cultures were maintained using Eagle's basal medium containing 5% inactivated horse serum. Penicillin in a concentration of 100 units per ml and streptomycin in a concentration of 100 μg per ml were included in all tissue culture media. When cultures were inoculated with respiratory secretions, 50 units of mycostatin and 100 μg of neomycin sulfate /ml were incorporated into the maintenance medium. All cultures were incubated at 36°C. *Virus strains.* The Long strain of respiratory syncytial virus, originally recovered by Chanock *et al.*(2,3), was brought by one of us (M.R.H.) to this laboratory from the Walter Reed Army Inst. of Research. *Antisera.* Acute and convalescent sera from a human case of respiratory syncytial virus infection, originally obtained from Dr. J. A. Morris, was brought to this laboratory from the Walter Reed Army Inst. of Research. Rabbit antiserum against the Long strain was obtained from Dr. R. M. Chanock. Paired sera from proved cases of respiratory syncytial virus infection were used for virus identification purpose. *Virus recovery.* HeLa or human amnion (WISH) cell cultures were inoculated, each in triplicate, with 0.25 ml amounts of the thawed samples of respiratory secretions from each patient. The cultures were observed daily for cytopathic effect for 9 days, being refed with maintenance medium every 3 or 4 days. A single blind passage was made of all cultures which were negative. Tubes showing a definite cytopathic effect were harvested and the contents frozen. One or 2 subsequent passages were necessary to provide enough material for identification. *Viral infectivity assays.* All virus infectivity titrations were performed using serial 10-fold dilutions of virus. The inoculum dose was 0.2 ml per tube containing 2.0 ml of maintenance medium. The titer was the highest initial dilution of virus causing 50% or more degeneration of the cell sheet by the seventh day post inoculation. *Complement fixation (CF) tests.* The diagnostic CF antigen consisted of fluids from HeLa cell cultures infected with the Long strain of respiratory syncytial virus which were frozen, thawed and harvested when the cell sheets were completely degenerated. Uninfected normal HeLa culture fluids, similarly prepared, were used for control purpose. Serial 2-fold dilutions of inactivated sera in 0.1 ml volume were tested using 4 units of antigen in 0.1 ml volume and 2 exact units of complement in 0.2 ml. Fixation was overnight at 4°C and the hemolytic system consisted of 0.2 ml of a 1% sensitized sheep erythrocyte suspension. The titer was the highest initial dilution of serum giving less than 25% hemolysis. The complement was standardized in the presence of antigen and was free of antibody against the respiratory syncytial virus. *Serum neutralization (SN) tests.* In the tests, serial 2-fold dilutions of the inactivated serum were incubated for ½ hour at room temperature with an equal volume of virus diluted to contain approximately 200 TCD_{50} per 0.2 ml. HeLa cell cultures, in duplicate, were inoculated with 0.2 ml of the virus-serum mixtures and the tests were read on the third or fourth day of incubation when the control tubes showed specific viral degeneration of 50 to 75% of the cell sheet. The titer was the

TABLE I. Diagnostic Test Results Obtained in Representative Cases of Respiratory Syncytial Virus Infection.

Patient		Laboratory findings				
		CF		Neutralization		
Age	Clinical diagnosis	Acute	Conv.	Acute	Conv.	Virus recovery
3 yr	Upper resp. illness	0*	320	0*	40	Positive
1 "	" " "	0	80	0	80	"
3 "	" " "	0	80	0	20	"
3 "	Bronchitis	0	320	0	160	"
3 "	"	0	160	0	20	"
2 "	"	10	320	0	80	"
6 mo	Bronchiolitis	0	40	0	10	"
8 "	"	0	40	0	20	"
1 yr	"	0	320	0	80	"
6 "	Bronchopneumonia	10	320	10	160	"
3 "	"	0	320	0	40	Negative
6 "	"	20	320	<20	640	"

* 0 titer = <1:5.

ighest initial dilution of serum which caused omplete suppression of viral degeneration. *dentification of viral isolates.* Isolates were dentified both by the CF and serum neutraization technics using paired sera from huıan cases of respiratory syncytial virus infecion, including the paired sera from the first eported human case of infection(1) with this irus. All identifications were controlled in imultaneous tests using the Long strain of espiratory syncytial virus. Tests with known ositive Myxovirus parainfluenza 1, 2 and 3, neasles and adenovirus group antisera gave egative results providing an additional conrol. *Results. Representative diagnostic est results.* Table I and Fig. 1 present repesentative serologic (CF and neutralization) nd virus recovery findings among 34 cases of respiratory illness caused by the respira-

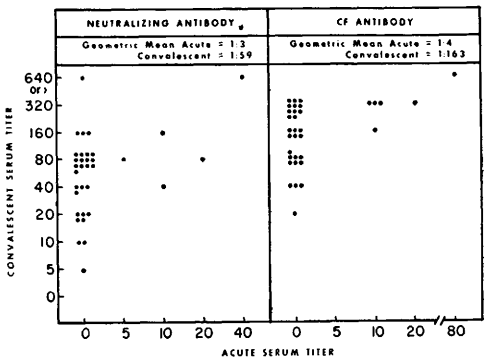

FIG. 1. Diagnostic neutralization and CF test findngs in paired sera from 34 cases of respiratory illness caused by the respiratory syncytial virus.

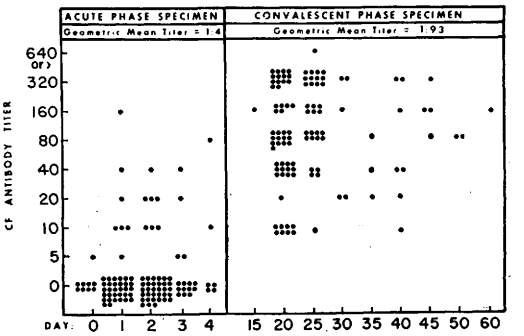

FIG. 2. Distribution, according to days post onset of illness, of CF titers in 109 cases of respiratory illness caused by the respiratory syncytial virus.

tory syncytial virus. These 34 cases were specially selected to include the syndromes of acute upper respiratory illness, bronchitis, bronchiolitis and bronchopneumonia. A single case of croup occurred among the cases but was not included in Table I. The acute phase serum specimens collected within 4 days of onset of clinical illness were usually devoid of neutralizing or CF antibody (< 1:5) although a few sera were positive initially with titers as great as 1:80. The convalescent serum specimens regularly showed a diagnostic (4-fold or >) increase in titer of antibody. The maximum fold-increase by neutralization was 256 or > and by CF was 128 or >. The geometric mean* acute and convalescent neutralizing antibody titers

* In computing the mean titers, a titer of < 1:5 was arbitrarily assumed to be 1:2.5.

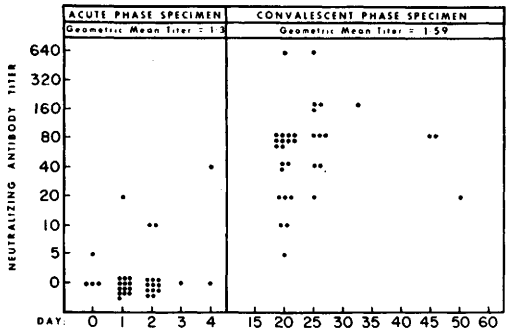

FIG. 3. Distribution, according to days post onset of illness, of neutralizing antibody titers in 34 cases of respiratory illness caused by the respiratory syncytial virus.

were 1:3 and 1:59, respectively, and mean CF titers were 1:4 and 1:163, giving mean fold-increases of 30 and 41, respectively. The serologic diagnoses usually could be confirmed by virus recovery.

Distribution of serum titers according to time post onset of illness. Fig. 2 summarizes acute and convalescent serum CF titers among 109 cases of respiratory illness diagnosed as respiratory syncytial virus infection based on the demonstration of a 4-fold or greater increase in CF antibody against the Long strain in tests with acute and convalescent sera. The geometric mean acute titer was 1:4 and mean convalescent titer was 1:93. The distribution of individual acute phase titers was roughly the same whether the specimens were collected on day 0, 1, 2, 3 or 4 post onset of illness. Maximal convalescent CF titers appeared to be reached by the fifteenth to twentieth day post onset of illness since titers of sera collected later were not appreciably different.

The findings in similar tests of neutralizing antibody in acute and convalescent sera from the 34 selected cases mentioned above are shown in Fig. 3. The geometric mean of the acute phase titers was 1:3 and of the convalescent was 1:59. In agreement with the CF results, the distribution of titer values in the acute phase specimens suggested no significant difference whether taken on day 0, 1, 2, 3 or 4 post onset of illness. Maximal neutralizing antibody titers appeared to be reached by day 20. It is of interest that a portion of the cases exhibited CF or neutralizing antibody in the acute phase specimen, indicating that infection, with illness, apparently can be superimposed on antibody resulting from prior infection.

Virus recovery results. An attempt was made to recover respiratory syncytial virus from respiratory secretions of the 34 selected cases diagnosed, positively, by the CF method. The distribution of virus recoveries, according to time post onset of illness, is shown in Table II. Respiratory syncytial virus was recovered from 21 of the 34 cases (62%). Recoveries were made on days 0-4 post onset of illness indicating that the virus is present for at least 4 days after appearance of clinical symptoms. The recovered isolates were identified as respiratory syncytial agent both by CF and by neutralization tests. In all instances, there was complete agreement in the results.

TABLE II. Distribution of Virus Recovery Results, According to Time Post Onset of Illness, among 34 Cases of Serologically Diagnosed Cases of Respiratory Illness Caused by Respiratory Syncytial Virus.

	Days post onset of illness specimen taken				
Result	0	1	2	3	4
Positive	3	9	8	0	1
Negative	1	6	4	1	1
% positive	(75)	60	67	(0)	(50)

Comparative sensitivity of diagnostic test methods. Table III summarizes results of diagnostic tests for 34 cases in which all 3 test methods were used. All 34 cases were diagnosed by CF and this result was confirmed by neutralization test findings in 33 cases (97%).

Respiratory syncytial virus was recovered from 21 of the 34 cases (62%). While the

TABLE III. Comparative Sensitivity of Various Diagnostic Test Methods for Detecting Respiratory Syncytial Virus Infections (Diagnosed Initially by CF).

Diagnostic test method	No. pos.	No. neg.	% positive
CF	34	0	100
Serum neut.	33	1	97
Virus recovery	21	13	62

virus recovery technic was the least sensitive of the 3 diagnostic tests, the number obtained is nonetheless an excellent experience for virus isolation results in general. It is important to note that 20 of the 21 virus recoveries were made in primary passage and that only 1 additional isolation was made by the routine subculture procedure. Further, only 1 or 2 of 3 inoculated tubes showed cytopathic effects on primary passage of some of the respiratory secretions.

Multiple serologic increases in antibody. Among the 563 respiratory disease cases studied, 19 additional persons showed a diagnostic CF titer rise against the respiratory syncytial virus plus adenovirus, Myxovirus parainfluenza 1, 2 or 3 or against influenza A_2. This indicated infection with more than 1 virus during the time period for the collection of the samples of blood. Adenovirus and parainfluenza 3 were encountered most frequently.

Discussion. These findings have clearly demonstrated the utility of both CF and serum neutralization tests for laboratory diagnosis of respiratory illness caused by the respiratory syncytial virus. Mean titers of the acute or the convalescent serum specimens were roughly the same when tested by the CF or serum neutralization technic and the two procedures appeared equally sensitive for detecting diagnostic increases in titer. These findings are at variance with those of Chanock *et al.*(2,3) who reported greater sensitivity of the neutralization technic, especially in infants and children in whom the neutralization test was stated to be twice as sensitive as the CF test. As a further test in our laboratory, paired sera from 13 cases of respiratory illness in very young infants 3 weeks to 5 months of age, collected at the height of a respiratory syncytial virus epidemic and found negative by CF, were tested by the neutralization method. The neutralization test results were also negative providing additional evidence that the CF test, properly performed, does not fail to detect cases.

The virus isolation test, while less sensitive than either of the 2 serologic procedures, was nonetheless highly efficient when appraised in terms of expected recoveries in virus diseases, generally. Thus, the virus was recovered in 62% of 34 selected cases of the disease. Previous workers(1,2,3,5) have experienced great difficulty in isolating respiratory syncytial virus from respiratory specimens which had been frozen. These workers did not state whether the samples had been handled to exclude carbon dioxide. One worker used 2% horse serum in Hank's solution and another used Hank's solution alone for collecting and storing the specimens. These are probably inadequate for preserving infectivity. In the present series, the specimens had been stored frozen in veal infusion broth up to 1 year prior to testing. The data were insufficient to permit definitive appraisal of the relative efficiency of HeLa and human amnion cell cultures (WISH) for virus recovery. It was observed, however, in cultures inoculated with the same virus preparation, that the HeLa cells degenerated 1 or 2 days earlier than did the amnion cells.

The present findings, like those of Beem *et al.*(5), present convincing evidence for the role of respiratory syncytial virus in cases of human respiratory illnesses. Thus, the virus is present in the acute illness at time of the first visit to the physician and the patient develops high levels of both neutralizing and CF antibodies during convalescence. Additional support for etiologic role of this virus is presented in the second paper in this series(6) in which very low prevalence of infection is demonstrated among well controls.

The clinical illnesses which occur in infection with the respiratory syncytial virus include mild acute upper respiratory illness, bronchitis, bronchiolitis, bronchopneumonia and, infrequently, croup.

Summary. Laboratory diagnostic findings in 109 cases of respiratory illness caused by the respiratory syncytial virus are presented. The CF and serum neutralization tests were equally sensitive for detecting cases and antibody titers obtained by the 2 methods were roughly the same. The virus isolation procedure was less sensitive giving positive results in 62% of the cases. Clinical illnesses associated with respiratory syncytial virus infection included acute upper respiratory ill-

ness, bronchitis, bronchiolitis, bronchopneumonia and, rarely, croup.

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Resistance of Polyoma Virus Immune Animals to Transplanted Polyoma Tumors. (26453)

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Previous reports(1,2), described establishment of transplantable lines of subcutaneous fibrosarcomas from tumors produced by inoculation of newborn animals with polyoma virus. Transplantable tumors in the hamster line and in one of the 2 mouse lines (C57-695) have never had demonstrable virus associated with them. The second mouse line (C57-1923) had virus associated with tumor for the first 10 transplant passages, but not in the subsequent 8 passages. Virus-negative tumors in tissue culture were susceptible to challenge with polyoma virus, and virus production could not be induced in them by irradiation. From this and the lack of demonstrable polyoma virus antigens in tumors, it was concluded that once virus had transformed normal cells to tumor cells it was no longer necessary for maintenance of the tumor.

As an additional piece of evidence for the above conclusion in studies of the hamster tumor(1), it was shown that adult hamsters made immune to virus and having demonstrable antiviral antibodies were still capable of supporting the transplantable tumor. However, this result was based on challenge with a large inoculum of minced tumor. The purpose of this report is to present evidence indicating that adult hamsters and mice made immune to polyoma virus do develop a resistance to transplantable tumors provided the challenge with tumor cells is made on a quantitative basis. Evidence is also presented that this resistance is specific and not due to serum antibodies.

Materials and methods. Animals. General purpose (G.P.) mice were random-bred Swiss, C_3H were C_3H/Hen and C57 were C57/Bl. Hamsters were random-bred Syrian hamsters. All animals were reared in the NIH animal colony. *Tumors.* The transplantable polyoma hamster tumor and C57-695 and C57-1923 transplantable polyoma mouse tumors have been described(1,2). The SW tumor was a fibrosarcoma originating in a C57/Bl mouse that had been inoculated with radioactive thorium and was kindly supplied by Dr. Richard Swarm of Nat. Cancer Inst. in its 16th transplant passage. The S180 tumor was maintained in the ascites form in G.P. mice and was originally obtained from Dr. Morris Belkin of the Cancer Institute. *Virus.* Polyoma virus grown in mouse embryo tissue culture had a titer of 10^7 ID_{50}/ml. *Hyperimmune rabbit serum.* Rabbits