this divergent behavior. In this context observations in abnormal infant lungs are of interest. It has been suggested, and some evidence presented(7) that in very small premature infants and in the respiratory distress syndrome the amount of surfactant in the lungs is reduced. If quantity of surfactant were the cause of the differences observed here, then these particular lungs should lose stability more readily than any others. Yet this is not the case either in very small infants (Fig. 2a), or in those with the respiratory distress syndrome (Fig. 2b). This is additional evidence that a qualitative diference exists between the newborn and the adult which causes the alveolar lining layer of the newborn to preserve its surface activity after repeated cycling of the excised lung while this is not so in the adult. The transition is gradual since intermediate stages have been observed (Fig. 1). The cause of this difference in behavior and presumably structure of the surfactant is unknown. The amount of phospholipid in lung tissue of the ewe is about ⅔ of that in her full term fetus for unit dry weight(8), even though it may be assumed that a greater potential surface area is contained in the sample from the adult per unit weight. It is known that the fatty acid content of the pulmonary phospholipid affects its surface activity. Therefore, it is pertinent to note that differences in serum cholesteryl ester fatty acids have been found between newborn and adult rats(9). It remains to be determined whether differences of this kind also exist in the surface active pulmonary phospholipid.

Summary. Excised lungs of human infants and 4 animal species were inflated and deflated at 2 to 8 cycles per minute. This resulted in a loss of stability of expansion in all adult and post-neonatal infant specimens tested, but was not true in neonatal lungs. It is suggested on the basis of available data that a qualitative as well as a quantitative difference exists between newborn and adult lungs in the surface active material of the alveolar lining layer. Intermediate stages have been observed in post-neonatal infant lungs of all species. Recovery of stability of expansion occurred at rest within a few minutes regardless of the state of expansion, or the use of air or nitrogen.

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Rubella Virus: Growth Characteristics and Stability of Infectious Virus and Complement-Fixing Antigen. (31143)

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Rubella virus specific complement-fixing (CF) antigen was recently prepared by Sever *et al*(1) using the "packed cell" method with continuous rabbit kidney (RK-13),* primary African green monkey kidney (AGMK), as well as other tissue culture systems. The data

* Originally obtained from Dr. J. A. Dudgeon, Hospital for Sick Children, London, England.

presented here extend the previous observations and compare the growth characteristics, physical properties and stability of both the infectious virus and the CF antigen.

Materials and methods. Virus and cell cultures. The RV strain(2) of rubella virus adapted to the continuous rabbit kidney (RK-13) cell line was used in these studies. This virus had 9 passages in African green monkey kidney tissue culture, 8 passages in RK-13 cells, and a titer of 5.4 $\log_{10}$/0.1 ml. The media for growth and maintenance of RK-13 cells have been described previously (1); however, the maintenance medium was modified by addition of 2% inactivated fetal calf serum. Primary tissue cultures of African green monkey† *(Cercopithecus aethiops tantalus)* kidney from commercial sources were used for virus titrations.

Virus titrations. Infectivity titrations of rubella virus in primary African green monkey kidney tissue cultures were determined by a modification of the enterovirus interference technique as described by Schiff *et al*(3). Briefly, serial 10-fold dilutions of virus samples were made in Eagle's basal medium and 0.1 ml of each dilution was inoculated into a set of 3 tissue culture tubes. Ten days after infection the overlay fluid from each infected tube was removed and replaced with fresh maintenance medium containing 100 $TCID_{50}$ of Coxsackie A-9 virus. Cultures were examined microscopically after 96 hours for cytopathic effects of Coxsackie virus. The absence of enterovirus cytopathic effect was interpreted as indicating the interfering effect of rubella virus. Fifty per cent interference (infectivity) endpoints were calculated by the method of Reed and Muench(4).

Complement-fixation (CF) test. The microtechnique CF test as described by Sever *et al* (5) using spiral loops and disposable plastic plates was used. Antigen titrations were carried out in two-fold dilutions and with 0.025 ml volumes. Acute and convalescent human sera from proven cases of rubella virus infection were used in the CF tests. Sera were inactivated at 56°C for 30 minutes. The diluent was veronal buffered saline as described by Kabat and Mayer(6).

Extracellular, intracellular virus infectivity and CF antigen during virus multiplication. Confluent RK-13 tissue cultures between passages 141 and 155 were grown in 32 oz (surface area 135 sq cm) bottles. The maintenance medium was removed and the cultures were inoculated with 8 ml of seed virus and incubated at 34°C for 30 minutes, during which time they were slightly agitated at intervals. The fluid overlay from the cultures was then removed and the cells washed 3 times with warm (34°C) maintenance medium. Fresh maintenance medium (40 ml per bottle) was replaced and the bottles reincubated at 34°C. At intervals of 2, 6, 24, 72, and 120 hours, infected and control cultures were removed. The supernatant fluid from each group was pooled and treated separately. The cultures were washed with 10 ml of cold (4°C) sterile veronal buffered saline. The cells were scraped from the bottles with a rubber spatula, resuspended in veronal buffered saline (1 ml per bottle). These cell preparations were centrifuged at 600 *g* for 10 minutes in a refrigerated (4°C) centrifuge. Packed cells were resuspended in supernatant veronal saline to give a 20% suspension. The 20% resuspended cells and the original pools of infectious fluid overlays were frozen in acetone-dry ice mixture and thawed 3 times. The resuspended cell preparations constituted the "packed cell" rubella CF antigen. The levels of infectious virus in the packed cells (intracellular virus), and the infectious overlay fluids (extracellular virus) were determined. All preparations were stored at −90°C until tested.

Temperature stabilities of virus infectivity and CF antigen. Infectious overlay fluids with rubella virus were first centrifuged at 6000 *g* for 30 minutes at 4°C to remove debris. Aliquots were subjected to various temperatures for specified periods in order to determine the stability of the infectious virus. Temperature stability tests were held within ± 2°C. The temperatures and maximum times used were: 100°C for 10 minutes, 70°C for 10 minutes, 56°C for 30 minutes, 37°C and 4°C for 7 days each. Aliquots of virus

† Purchased from Microbiological Associates, Inc., Bethesda, Md., and Flow Laboratories, Inc., Rockville, Md.

samples were taken at various intervals, and immediately placed at —90°C until tested. Each virus preparation was inoculated into African green monkey kidney tissue culture cells and blind passed 3 times before being declared negative.

CF antigen detected in the intracellular preparation was tested for stability at 34°C for 72 hours. At 24-hour intervals samples were removed and frozen at —90°C until tested for CF activity.

Effect of ultraviolet irradiation on infectivity and CF antigen. The method of ultraviolet irradiation used has been described(7). Briefly, clarified preparations of infected tissue culture fluids or "packed cell" CF antigens centrifuged at 1500 rpm for 15 minutes and filtered through a millipore filter (0.88 μ) were placed in open petri dishes at a distance of 19 cm from the ultraviolet light. Filtration did not affect the infectivity titers or the CF property. Samples were slightly agitated manually during the periods of UV exposure. At intervals of 1, 3, 5, 10, 15, and 30 minutes, aliquots of CF antigen were removed and kept frozen at —90°C until tested. For determination of stability of infectivity, aliquots were taken at intervals of 20 seconds, 40 seconds, one, 3, and 5 minutes and kept frozen at —90°C until tested.

Each experiment described was repeated 3 times. The results reported herein show representative data obtained from these experiments.

Results. The amount of virus detected in the maintenance fluid overlay of RK-13 cells 30 minutes after infection was equivalent to 50% of the virus inoculated. This indicated that only 50% of the virus input was absorbed and was responsible for initiating infection of the rabbit kidney cells. Intracellular and extracellular infectious virus decreased for 6 hours after infection (Fig. 1). The titers then increased for 48 hours in the intracellular material and for 72 hours extracellularly. The highest titer was 6.8 $\log_{10}$/0.1 ml which occurred in the intracellular material 48 hours after inoculation. The increases in virus were followed by plateaus which lasted until the experiment was terminated. The increase in titer was more rapid and persisted longer in the intracellular material. Typical rubella cytopathic degeneration in infected RK-13 cells was noted at 120 hours (8). All control uninfected cells did not contain detectable levels of either intracellular or extracellular infectivity.

Complement-fixing intracellular antigen was first detectable 48 hours after infection (Fig. 1). The peak titer of CF antigen was attained at 72 hours followed by a decrease at 120 hours. Since no detectable CF antigen was present in the extracellular fluid(1), tests on the supernatant fluids were not carried out.

The results on thermal stability of rubella virus infectivity show that at 4°C, rubella virus was stable for at least 7 days. At 37°C virus infectivity was inactivated to below detectable levels by the end of 48 hours; at 56°C it was inactivated within 30 minutes; this inactivation followed a first order reaction. At temperatures of 70°C and 100°C, inactivation of virus infectivity was accomplished within 4 and 2 minutes, respectively.

The stability of the CF antigen at 34°C was tested to determine if the fall in CF titer as noted in the growth curve (Fig. 1) after 72 hours' incubation could be partially due to temperature inactivation. The results of these tests (Fig. 2) established that at 34°C, eight units of complement-fixing antigen were destroyed within 72 hours. The rate of inactivation of the CF antigen also followed a first order reaction.

The effects of ultraviolet irradiation on infectivity and the CF antigen are shown in Fig. 3. CF antigen was not affected by as long as 10 minutes' exposure to ultraviolet rays, while extracellular virus infectivity was reduced below detectable levels with 40 seconds' exposure. Ultraviolet inactivation of the virus infectivity followed a first order reaction, as did thermal inactivation. Studies of infectious intracellular virus in the "packed cell" CF antigen materials showed that live virus in these preparations was not completely inactivated after 30 minutes' ultraviolet irradiation. Infectivity titers in these samples fell from initial levels of 7.2 to 1.5 ($\log_{10}$)/ml. The protective effect of extra protein in the CF antigen containing infec-

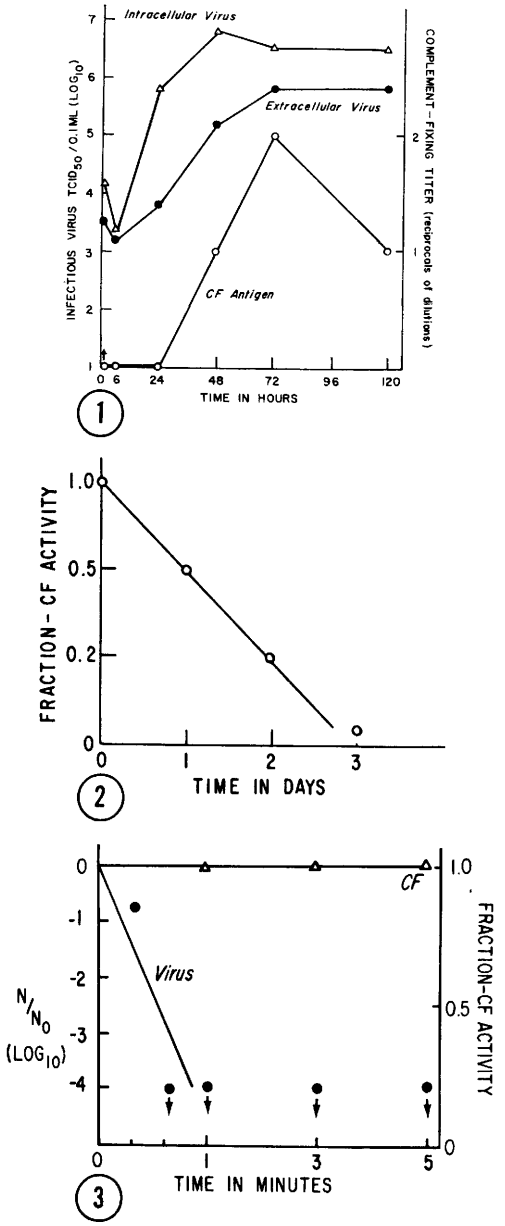

FIG. 1. Appearance and levels of intracellular, extracellular virus infectivities and cell associated CF antigen during rubella virus multiplication in continuous rabbit kidney (RK-13) tissue culture cells. Arrow marks levels of infectious virus 30 min post-infection.

FIG. 2. Stability of rubella virus "packed cell" CF antigen at 34°C. Antigen used had an initial CF titer of 1:8. Results are expressed as fractions of residual CF titer, where original titer has an arbitrary value of one.

FIG. 3. Effect of ultraviolet irradiation on rubella virus infectivity and CF "packed cell" antigen. N/N_0 = proportion of residual infectivity (N_0 = initial infectivity titer, N = infectivity titer after treatment). See also note Fig. 2.

tious virus offers a reasonable explanation for the residual infectivity in the "packed cell" antigen.

Discussion. The results in the growth studies in RK-13 tissue cultures show that the levels of intracellular infectious virus were consistently higher than those of the extracellular virus. The higher level of intracellular virus may be related to the accumulation of virus within the cell; matured virus ejected into the medium would naturally be diluted. Similar findings were reported by Parkman *et al*(9) when low but not high multiplicities of infection were used in African green monkey kidney tissue cultures; however, the titers attained with this tissue were lower than those in the RK-13 tissue.

Rubella virus CF antigen was first detected 24 hours following inoculation of the cultures and thereafter increased in parallel with infectious virus until 72 hours, at which time CF antigen fell while infectivity levels remained unchanged. The data on inactivation of the CF antigen at 34°C indicate that the loss of CF antigen in cultures after several days can be accounted for by heat inactivation. Some of the CF antigen may also be lost by release from the cells and dilution in the culture fluid. Sufficient data, however, are not yet available to determine whether the CF antigen is a subunit of the infectious virus or a non virion cell antigenic component synthesized during infection.

The decrease in infectivity of rubella virus with exposure to heat or ultraviolet irradiation follows the curve of a first order reaction. Both the CF antigen and the virus, however, showed similar rates of inactivation at 34°C and 37°C respectively.

Summary. The developments of infectious virus (extracellular and intracellular) and the specific CF antigen of rubella virus were studied in a continuous line of rabbit kidney tissue culture cells. The thermal stability and effect of ultraviolet irradiation on the virus and the CF antigen were also examined. During initial virus synthesis, intracellular infectivity titers were consistently higher than the

extracellular titers. Intracellular infectivity reached a peak titer of 6.8 $\log_{10}$/0.1 ml at 48 hours after infection, after which it fell slightly and leveled off. Extracellular virus infectivity did not reach its peak titer of 5.8 $\log_{10}$/0.1 ml until 72 hours after infection, and remained stable until termination of the experiment. Rubella CF antigen was first detected 48 hours after infection and reached a maximum titer at 72 hours, followed by a sharp decrease. This decrease in titer was attributed to a probable stop in synthesis of the antigen by the infected cells, and thermal inactivation. Thermal and UV inactivation of virus infectivity followed the expression of a first order reaction. The CF antigen was thermo-labile at 34°C, and its inactivation was also exponential; the antigen was, however, stable to ultraviolet irradiation up to 30 minutes exposure.

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ATP Concentration and Localization of Sites of Epinephrine Induced Renal Artery Constriction.* (31144)

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Segmental constriction of the dog's renal artery in response to pharmacological doses of epinephrine has been described(1). This constriction in the main renal artery appeared predominantly in the zone of the first major bifurcation, occasionally in the mid portion, and less frequently in the proximal segment. Repeat studies in the same animal had demonstrated the reproducibility of this epinephrine induced constriction. Similarly, localized segmental constriction in response to pharmacological doses of epinephrine has been observed in the common, internal carotid and vertebral arteries. A narrowing of the post renal aortic segment has also been observed.

Histological studies of serial sections along the renal artery have revealed no morphological difference which could account for the observed zonal constriction. Consequently, studies of biochemical properties and functions of zonal segments of the renal artery have been initiated in an effort to see if any variation in these properties along the renal artery could be correlated with segmental renal artery constriction.

The intensity and prolonged time of the localized segmental constriction of the renal artery in response to epinephrine led us to hypothesize that such segments would require higher energy stores (adenosine triphosphate concentration—ATP) and/or higher ATP

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