epinephrine was studied. Intravenous injection of even 0.2 μg/kg of the food poisoning toxin induced a significant increase in the vasoconstrictive effect of topically applied epinephrine within 3 hours. Maximum sensitivity occurred about 10 hours after toxin administration and persisted over 18 hours with toxin levels of 1 to 10 μg/kg. A rapidly developing state of hyporeactivity to epinephrine resulted from much larger enterotoxin challenges. Except for the much slower evolution of the state of increased responsiveness to epinephrine, these results are similar to those induced by bacterial endotoxin.

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Density Gradient Centrifugation Studies of Rubella Virus. (31162)

C. L. CUSUMANO (Introduced by R. J. Huebner)

Section on Infectious Diseases, Perinatal Research Branch, National Institute of Neurological Diseases and Blindness, Bethesda, Md.

Little information is available on the physical and chemical characteristics of rubella virus. Norrby *et al*(1) reported that the virus could not be sedimented when placed on a gradient in which the lowest density was 1.08 g/cm^3. Others (Sever *et al*(2); Parkman *et al*(9); Cusumano *et al*(5)) have published data on the size, nucleic acid, and sensitivity to heat and chemical agents of this virus. A major impediment to more detailed physicochemical study of this virus has been the fact that rubella titers in tissue culture are only in the range of 2 to 4 Log_{10} $TCID_{50}$/0.2 ml, and rarely 5 Log_{10} $TCID_{50}$. The recent availability of techniques for rapidly concentrating large volumes of virus suspension has permitted the practical use of density gradient centrifugation in the study of this virus.

Materials and methods. Virus. Rubella virus was grown in each of 3 cell lines. Two strains of virus were used. The RV strain (Sever *et al*(2)) was isolated in this laboratory and was passed 14 times in African green monkey kidney (AGMK) cells. A subline of the RV strain rubella virus was propagated in RK-13 continuous rabbit kidney cells (McCarthy *et al*(8)). A third rubella virus preparation was obtained from a chronically infected strain of LLC-MK_2 continuous rhesus monkey kidney cells (Brown *et al*(3)).* It should be noted that all known rubella virus strains are serologically indistinguishable (Sever, J. L.—unpublished data).

Monolayers of each infected cell line were grown in 32 oz bottles according to previously described methods (Sever *et al*(2,4)); McCarthy *et al*(8)) and where necessary were inoculated with rubella virus. Fluids from infected and control cultures of each cell line were harvested three times a week during the life of the culture and were stored at −70°C until used. All virus assay procedures were conducted in AGMK tissue cultures as previously described (Sever *et al*

* Originally provided through the kindness of Dr. H. Maassab, Univ. of Mich.

(2)), using 3 tubes per dilution. Endpoints were calculated by the Reed-Muench method.

Concentration of virus. After centrifugation at 1000 rpm for 20 minutes, tissue culture fluids were concentrated by vacuum dialysis with an LKB 6300 A Ultrafilter (LKB Produkter, Bethesda, Md.), or by pressure dialysis with a #80—0010 Pressure Dialysis Cell (National Instrument Laboratories, Inc., Rockville, Md.). Concentrates were clarified by centrifugation. With either procedure theoretical rises in titer were routinely achieved. The presence of 1% serum in the LLC-MK_2 and RK-13 fluids limited concentration to approximately 100-fold. With the virtually serum-free AGMK preparation, concentrations of 800- to 900-fold were obtained.

Centrifugation. The SW-39 rotor of the Spinco Model L was used for all gradient experiments. Gradients were made by layering in succession 1.5 ml each of 40% and 25% sucrose and 1.7 ml of 5% sucrose. It was found experimentally that these concentrations formed uniform, steep, reproducible gradients in the region of the virus band, even when the tubes were centrifuged immediately after preparation. The virus concentrate was layered on top of the gradient in a volume of 0.2 to 0.3 cc/tube. Gradients were centrifuged at 39,000 rpm for 18 to 24 hours and the rotor was allowed to coast to a stop. Fractions of 15 drops each were collected into tubes containing 0.3 ml of Hanks' solution. Every fifth fraction was collected without Hanks'. The fractions were stored at —70°C. Each diluted fraction was assayed for virus infectivity and its optical density at 260 and 290 mμ was measured in a Beckman Model DU spectrophotometer. The undiluted fractions were used for refractive index determinations in a B & L Abbe 3-L refractometer and densities were calculated from a refractive index-density curve: Density $= \eta^{25}_{D} -$ 0.9621/0.3715.

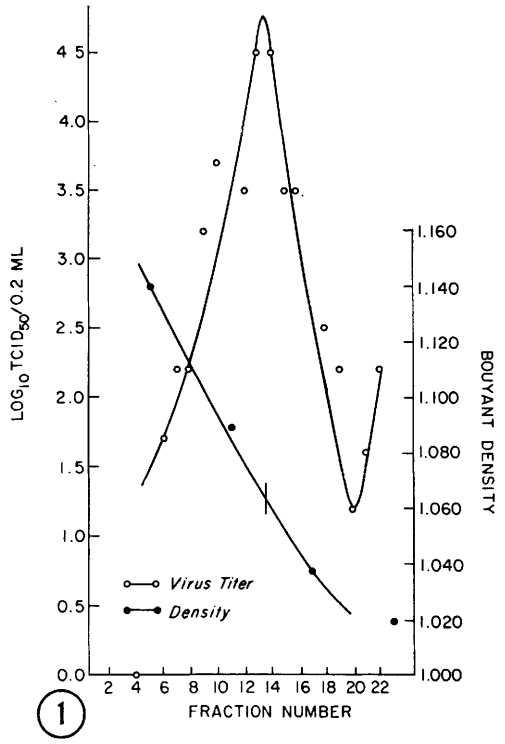
An experiment was designed to separate the double infectivity peaks observed in certain tests by sedimenting the virus in a medium whose density was midway between the peaks. Sucrose was dissolved in a 25 times concentrated LLC-MK_2-derived virus preparation to a concentration of 16%, and the resulting solution was sedimented for 15 hours at 35,000 rpm in a Spinco #40 head. The upper, middle, and lower thirds of the supernate were collected by needle punctures in the side of the tube, and the pellet was resuspended in 0.5 cc of Hanks' BSS. Each of these preparations was titered, centrifuged on the usual sucrose gradient, and analyzed as described above.

TABLE I. Density Peaks and Density Ranges of Rubella Virus on Sucrose Gradients.

Cell line for growth	Density of infectivity peak(s) (g/cm³)	Titer of peak(s) (log_{10} $TCID_{50}$)
(a) Concentrated virus banded on gradient		
RK-13	1.045	1.5
	1.075	.7
	1.036	1.5
	1.075	1.0
	1.080	3.5
LLC-MK_2	1.052	3.0
	1.075	3.0
AGMK	1.068	4.5
	1.075	
(b) LLC-MK_2 concentrate after sedimentation. Pellet and supernate fractions banded on gradients.		
Upper 1/3	1.062	2.7
Lower 1/3	1.073	4.0
Pellet	No peaks	

Results. (Table Ia). Virus infectivity curves from gradient studies formed well defined peaked bands with 99% of the infectivity present between 1.040-1.120 g/cm³ (Fig. 1). In 5 preparations a peak was consistently found between 1.068 g/cm³ and 1.080 g/cm³ with the average being 1.075 $\pm$ 0.005 g/cm³. Certain preparations of RK-13 and LLC-MK_2 materials exhibited, in addition, second infectivity peaks at values of 1.040, 1.045, and 1.053 g/cm³ in different runs (Fig. 2).

In the test designed to separate the double infectivity peaks, the sedimentation run resulted in the concentration of most of the virus in the pellet (Table II). When the fractions from this test were examined on sucrose gradients, a number of effects were noted (Table Ib). Virus from the upper 1/3 yielded narrow bands with single peaks at 1.070 to 1.079 g/cm³ (Fig. 3). Material isolated from the lower third of the sedimentation tube



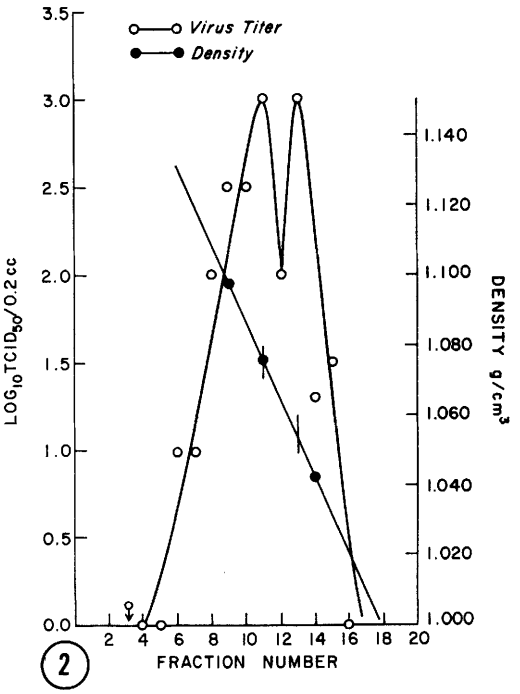


FIG. 1. Band with single peak observed in concentrated AGMK derived virus.
FIG. 2. Band with double peak observed in concentrated LLC-MK$_2$ derived virus.

yielded bands which had the usual 1.070 ± 0.005 g/cm^3 peaks, but which were markedly skewed towards the high density side as far as 1.175 g/cm^3. When the resuspended pellets were tested on gradients, they were found as irregularly varying levels of titer without peaks at densities ranging from 1.050 to 1.213 g/cm^3. In addition, the maximum titers found in the recentrifuged pellet gradients were reduced by over 4 Log_{10} $TCID_{50}$ below the titer of the resuspended pellets (Table II). In contrast to this finding, the routine centrifuge runs had maximum titers reduced by about 0.5 to 1.0 Log_{10} $TCID_{50}$ below the value found in the original virus concentrate.

Centrifuged gradient tubes all presented a similar appearance and no gross differences

TABLE II. Titers of Fractions from the Sedimentation Procedure (Log_{10} $TCID_{50}$).

	Sample A	Sample B
Upper 1/3	2.7	3.0
Lower 1/3	4.0	4.5
Pellet	4.7	5.2

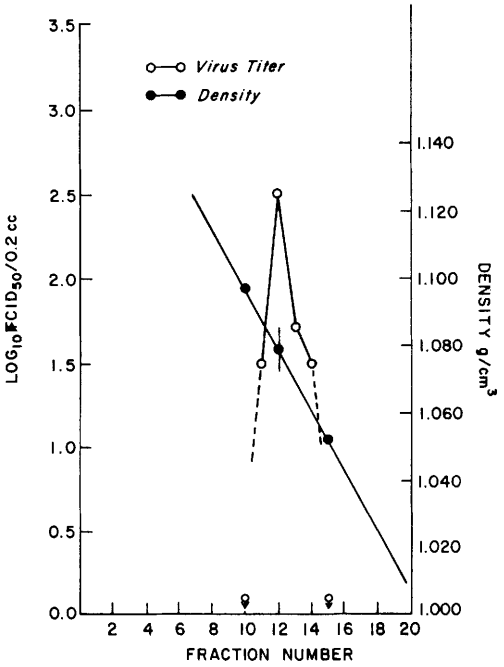


FIG. 3. Top fraction from sedimentation study using concentrated LLC-MK$_2$ derived virus.

were observed which distinguished the virus tubes from the controls. A diffuse yellowish band was noted in the upper ⅓ of all tubes.

Spectrophotometric readings revealed a dense, broad absorption band at both 260 mμ and 290 mμ in infected and control LLC-MK_2 and RK-13 preparations. The absorption peaks did not correspond with virus infectivity peaks in these preparations and optical densities 260 and 290 mμ were approximately equal. In AGMK preparations optical density peaks at 260 mμ and 290 mμ corresponded exactly with the virus infectivity peak in the virus-containing tubes but they were also present in the control tubes at the same density.

Discussion. Infectious virus was equilibrated at a density considerably lower than those published for other ether-sensitive (Sever *et al*(2)) viruses. This implies that the virus has an unusually high lipid content. In addition to, or alternative to, this hypothesis, the virus particles could be associated with a significant amount of water which might either be trapped within the viral envelope or bound to its surface. Either way, a considerable proportion of the migrating particle would consist of low density material.

Low density could result from adsorption of the virus onto subcellular lipoprotein fragments. However, one would not expect this process to occur to the same degree or to give the same density in three different tissue culture systems.

Second peaks which occurred at lower densities in some preparations were eliminated by centrifugation in an isodensity medium. Only the peak at 1.075 ± 0.005 g/cm³ remained in the supernatant virus after this treatment. Such lower density peaks apparently represent a reversible phenomenon such as adsorption to lipoprotein particles or to the wall of the tube, and do not reflect the presence of two classes of particles.

Virus particles which underwent more severe conditions of hypertonicity or centrifugal force, *i.e.*, those in the pellet or near the bottom of the sedimentation tube, were found to have an increase in heavy components when examined on a gradient. This phenomenon is similar to the behavior of Rauscher virus when it is subjected to prolonged centrifugation (O'Connor *et al*(7)). Probably the virus particles lose a part or all of their lipid content when subjected to this type of stress and therefore become more dense. The pellet material completely lost its usual density-distribution characteristics: the range over which it was found was shifted toward high densities; it did not form peaks; and its titer was reduced by 3 to 4 Log_{10} $TCID_{50}$ below the control values. This behavior implies marked disruption and loss of part of the viral lipid content. In addition, it is possible that some viral infectivity was lost in aggregates which sedimented to the bottom of the gradient tube.

The present observations are consistent with the view that rubella virus, an RNA virus (Cusumano *et al*(5)), is physically similar to the so-called "membrane viruses" (Crawford(6); O'Connor *et al*(7)).

Summary. Rubella virus was grown in three different mammalian kidney cell lines. The resulting virus-containing supernates were concentrated by forced dialysis and were then studied for density on sucrose gradients. All of the virus preparations produced peaked infectivity bands with 99% of the infectivity concentrated in a band from 1.040 to 1.120 g/cm³. The peaks had an average density of 1.075 ± 0.005 g/cm³. Possible reasons for this low density are discussed. Severe conditions of centrifugation caused the formation of additional infectivity peaks at higher densities. Pelleted virus formed a very broad, low titered infectivity band without peaks. The results are similar to those obtained with other RNA lipid-containing viruses and support the view that rubella virus is physically similar to them.

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Absence of Adrenocortical Stress Responses in Experimental Pyelonephritis.* (31163)

ARTHUR YUWILER, EDWARD GELLER, SHAWN SCHAPIRO, AND LUCIEN B. GUZE

Neurobiochemistry Laboratory and Medical Service, Veterans Administration Center, Los Angeles, Neuroendocrinology Laboratory, Veterans Administration Hospital, San Fernando, and Department of Medicine and Psychiatry, UCLA Center for the Health Sciences

Infection is a primary threat to life and higher organisms have developed an intricate set of responses to combat invaders and repair resultant tissue damage. By their very nature such responses represent homostatic disturbances likely to elicit corresponding compensatory physiological reactions. Despite the relevance of such a disturbance and response to the theory of stress as an adaptive mechanism, adrenocortical activation has been studied in relatively few diseases and little is known about the adrenocortical stress response in various stages of infection. As Selye(1) has pointed out, such studies are of particular theoretical interest because of the ambiguous survival value of the adrenocortical response in the infectious process. Indeed, the very anti-inflammatory properties of the adrenal corticoids which facilitate tissue repair and alleviate attendant symptomatology also are actions permitting spread of the infection and accentuate the danger to the host.

As part of a larger survey on enzymic and hormonal responses to stress, the present study concerns the endogenous adrenocortical response during stages of an infectious process. Experimental pyelonephritis was selected in this study for several reasons. The disease is readily produced in the laboratory rat by intravenous injection of *Str. faecalis* and its natural history has been studied by bacteriological(2), immunological(3), histochemical (4), and physiological(5) techniques. Injected bacteria are rapidly cleared from the blood by the liver and spleen and are destroyed in these organs. The renal microbial population, however, increases shortly after infection and then remains relatively constant throughout the life of the animal (2+ years). During this time, the infection evolves histologically from an acute phase with abscesses, tissue destruction and polymorphonuclear cell infiltration, through a subacute, to a chronic stage with tissue repair, scarring and mononuclear cell infiltration. The earliest lesions seen are microabscesses in the medullary portion of the kidney 2-3 days after injection. Functional changes occur early and are primarily manifested by an impaired concentrating ability. Clinically, the animal does not appear ill. Indeed, the rat eats normally and rate of weight gain is unaffected(6). Thus, in its insidious chronicity, histological appearance and early functional changes, the disease is similar to renal infection in man.

This model, therefore, permits evaluation of adrenocortical activation accompanying progressive tissue damage without introducing extraneous "non-specific" stress factors such as hyperthemia, anorexia, and anoxia. In addition, the close similarity between the experimental and natural diseases suggested also

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