meability (12) and make even greater demands upon the limited energy reserves. When both respiration and glycolysis are inhibited (by a different technique) in mouse soleus muscle (13), the membrane potential falls precipitously within 30 min. The obvious conclusion from our data as applied to that of others is that mammalian skeletal muscle is at a greater disadvantage than amphibian skeletal muscle whenever a diminished metabolism is experienced.

Summary. The "basal" rate of energy utilization, as measured by PC disappearance, in mouse anterior tibial muscles exposed to a N_2–IAA environment is about 0.25 μM/gm per min or 2.5 mcal/gm per min. This rate, as expected, is greater than the "basal" value for amphibian skeletal muscle and less than the "basal" value for mammalian cardiac muscle. By making certain assumptions about active ion transport and by using certain data from other investigators, the conclusion is reached that mammalian skeletal muscle sustains itself with a restricted energy supply much less well than amphibian skeletal muscle.

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Density Gradient Ultracentrifugation of Rubella Virus (32917)

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The development of a hemagglutination test provided a sensitive tool for defining the properties of rubella virus. Some of the biological and biophysical characteristics of rubella virus hemagglutinin (HA) were described in earlier reports from this laboratory (1–3).

Using sucrose, cesium chloride (CsCl) and cesium sulfate (Cs_2SO_4) density gradient techniques, it has been possible to band rubella virus HA and infectivity, and to define the buoyant density of rubella virus. These studies have enabled us to demonstrate the similarity of rubella virus strains grown in several mammalian cell systems and harvested at various passage levels. These experiments indicate that the sedimentation behavior of the HA and infectious virus are closely related.

Materials and Methods. Cells. The BHK 21 (4), BS-C-1 (5), and primary African green monkey kidney (GMK) cell cultures were obtained from the Tissue Culture Section, Division of Biologics Standards, Nation-

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al Institutes of Health. Eagle's minimal essential medium containing 2% heat-inactivated (56°C, 30 min), kaolin-treated, fetal bovine serum was used as maintenance medium (1).

Virus. Rubella virus, strain M-33 (6) was used in sixth and one hundred twenty-first GMK passage and third BS-C-1 passage. Strain Putnam (7) had been passaged 12 times in RK_{13} (8), once in MA-104 (Microbiological Associates) and twice in BHK 21 cell cultures.

Hemagglutinin. In most experiments, HA preparations represented unconcentrated supernatant fluids harvested from infected tissue cultures. These fluids were clarified either by centrifugation at 1000 *g* or filtration through 450 mμ Millipore filters. For certain experiments antigens were prepared by sonicating 5 ml at 4°C, at full power for 2 min, using a Bronson Sonifier. Antigens were also treated with polyoxyethylene sorbitan monooleate (Tween-80) and ether as described by Norrby (9). Rubella virus HA prepared in GMK or BS-C-1 cells was first concentrated by concentration at 96,000 *g* for 90 min. The lower one fifth of the centrifuged tissue culture fluid was then treated with Tween-80 and ether.

The HA titrations were performed in large disposable plastic plates. Serial 2-fold dilutions of antigen were made in pH 7.0 dextrose–gelatin–veronal buffer containing 0.2% bovine plasma albumin (1). An equal volume of 0.22% day-old chick red cells was added and the titrations were incubated at 4°C. The test was read after 24 hours and the HA titer expressed as the last dilution showing any indication of red cell hemagglutination. The specificity of each HA preparation was determined with known positive and negative antirubella sera.

Infectivity titrations. Rubella virus infectivity assays were performed by the interference method in GMK cultures using echovirus type 11 for challenge (10). The 50% interference dose (InD_{50}) was calculated according to the method of Karber (11).

Sucrose centrifugation. Preliminary experiments were performed using linear sucrose gardients from 5–35%, 15–45%, and 30–58%. Zonal centrifugation studies also were done in single sucrose concentrations of 6, 12, 15, 30, and 35%. One-half ml each of untreated and treated (Tween-80, ether) rubella virus HA was layered over 4.6 ml of sucrose and centrifuged in the SW39 Spinco rotor at 4°C for periods varying from 30 min to 20 hours, at an average force of 96,000 *g*.

CsCl and Cs_2SO_4 centrifugation. Linear 37–58% gradients of CsCl (General Biochemicals and American Potash Co.) were prepared, and 1.0 ml of rubella virus HA was layered over 4.6 ml of gradient. These preparations were also centrifuged in the SW39 Spinco rotor at 4°C from 45 min to 48 hours at 96,000 *g*. The bottom of the centrifuge tube was pierced with a 27-gauge needle and 5-drop fractions were collected into sterile cups for HA testing; 0.1 ml of each fraction was removed for infectivity titrations.

The Cs_2SO_4 (Fisher Biochemicals) gradients were processed identically except that linear 20–60% gradients were used.

Densities were calculated from standard curves prepared for each batch of CsCl and Cs_2SO_4. A Bausch and Lomb hand refractometer was used for all determinations.

Results. Preliminary experiments employing sucrose indicated that rubella virus HA (Tween-80 ether-treated, untreated, or a mixture of these two) appeared in a broad band in the midportion of the 30–58% gradient after 4 hours of centrifugation. Furthermore, narrow bands were not obtained after centrifugation for as long as 20 hours at 96,000 *g*. Of 50 fractions collected from these gradients, 37 contained both HA and infectious virus. Rubella virus HA and infectivity were not separated by sucrose density gradient centrifugation.

It was not possible to separate untreated HA from Tween-80, ether-treated HA nor from infectivity under any zonal centrifugation conditions with sucrose.

Experiments using CsCl were undertaken in an attempt to obtain narrower bands and to assess more accurately the relatitionship between rubella virus HA and infectivity. The result of a typical experiment using a linear 37–58% CsCl gradient is illustrated in Fig. 1. These antigens, prepared in BHK cells

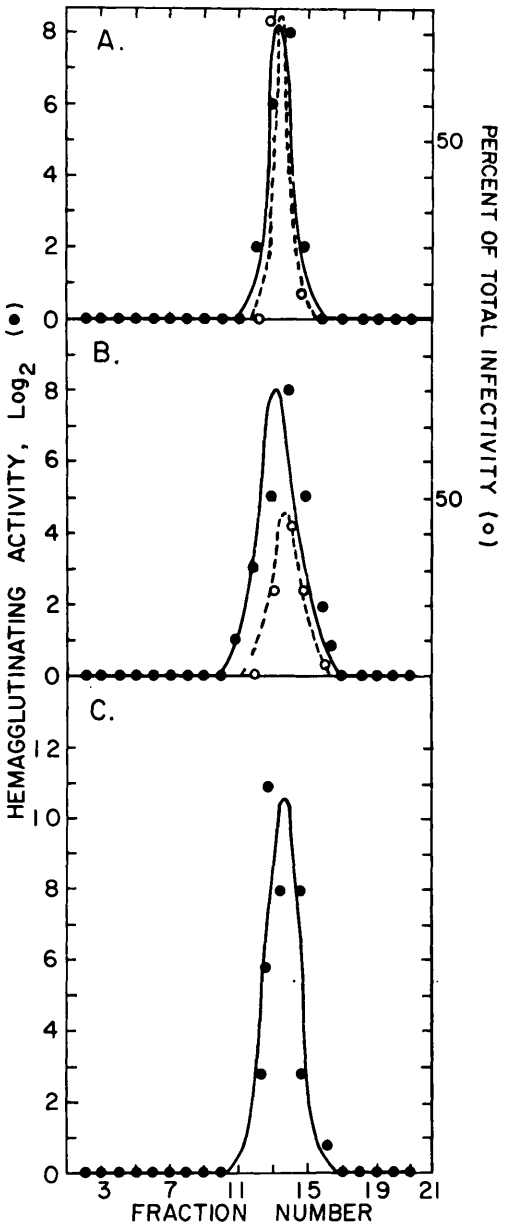

FIG. 1. Density gradient centrifugation of rubella virus HA in CsCl. A. Untreated antigen; B. Same antigen as A, but sonicated for 2 minutes; C. Same antigen as A, but Tween-80–ether treated. Each preparation was centrifuged in the SW39 rotor for 90 min at 96,000g ———, in A, and B, represents percentage of recovered infectivity. Statistical analysis has shown that normal curves fit these data well.

from the Putnam virus strain, were centrifuged for 90 min at 96,000*g*. There was no difference in sedimentation among any of the three preparations; the sonicated and Tween-80, ether-treated antigens produced a peak at the same level as the untreated HA antigen. As reported earlier (3) Tween-80, ether treatment often results in increased HA activity while virus infectivity is destroyed; here, the HA titer increase is reflected in a rise in peak titer from 1:256 to 1:1024. Maximum infectivity titers occurred at the same point as rubella HA; greater than 99% of the recovered infectivity was found in the same fractions. Typically, 6 $\log_{10}InD_{50}$ per ml of rubella virus was present at the peak of infectivity in CsCl gradients. While in some experiments infectivity was reduced 100–1000 fold, in others there was less than 20% loss. This variation is presently under investigation.

While rubella HA and infectious virus were very well localized in the gradients, visible bands were not present when the cellulose nitrate tubes were observed with either oblique or direct lighting.

Data presented in Fig. 2 show the localization of rubella HA prepared in GMK, BS-C-1, and BHK 21 cells. Maximum levels of HA activity were all observed at the same apparent density in the CsCl gradient; thus the type of cell culture used for antigen production did not alter HA sedimentation. These experiments also indicated that rubella virus HA from the one hundred twenty first passage level, representing attenuated, high passage virus, was concentrated at the same density as third, sixth, and fifteenth passage level viruses.

The HA titers of these rubella virus preparations are shown in Table I. The BHK-passaged virus had a higher HA titer than the other preparations; only antigens prepared in BHK 21 vells showed HA activity in unconcentrated tissue culture fluids. It is apparent from data in Table I that density gradient centrifugation of rubella virus concentrated the HA from 8- to 32-fold in the peak fraction.

To obtain data on the approach to equilibrium and the buoyant density of rubella virus HA, the Tween-80, ether-treated preparation was centrifuged on linear CsCl and

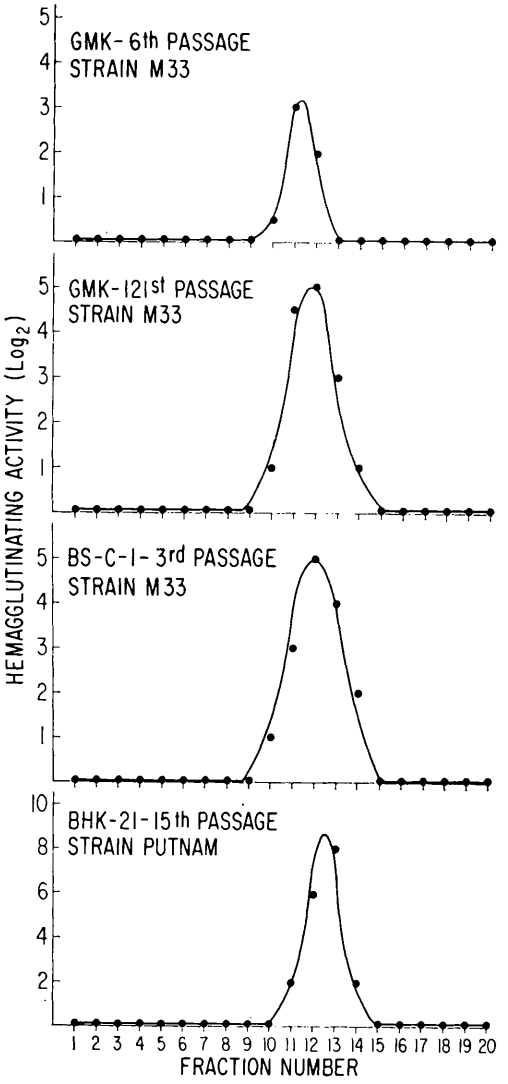

FIG. 2. Density gradient centrifugation of 4 different rubella virus preparations in CsCl. Each preparation was first concentrated by sedimenting the virus at 96,000g for 90 min. The sedimented virus was then treated with Tween-80–ether and centrifuged on a CsCl gradient. The source of each preparation is indicated on the Figure.

Cs_2SO_4 gradients for increasing time period until equilibrium was reached. Figure 3 illustrates the isodensity point of rubella virus HA after the HA was sedimented in the Cs_2SO_4 gradients for 19 hours at 96,000*g*. Maximal HA activity was found at a density of 1.23.

The data presented in Fig. 4 demonstrate that the HA reached sedimentation equilibrium in Cs_2SO_4 after 4 hours of centrifugation in preformed gradients. In CsCl, a greater density value was found at all time periods, and equilibrium was not reached until 10 hours of centrifugation. At all time periods in both CsCl and Cs_2SO_4, a similar sharp band, as illustrated in Fig. 3, was obtained. Thus, at equilibrium in CsCl, the peak of rubella virus HA activity occurred at density 1.33; in Cs_2SO_4 the peak of activity appeared at

TABLE I. Concentration of Rubella Virus HA in CsCl Density Gradients.

Rubella virus			HA titer[a]	
Strain	Culture system	Passage level	Before conc.	After conc.
M33	GMK	6	<2	8
M33	GMK	121	<2	32
M33	BS-C-1	3	<2	32
Putnam	BHK-21	15[b]	8	256

[a] Reciprocal of hemagglutinin titer per 0.2 ml.
[b] Twelve passages in RK_{13} cells; 1 passage in MA-104 cells; and 2 passages in BHK-21 cells.

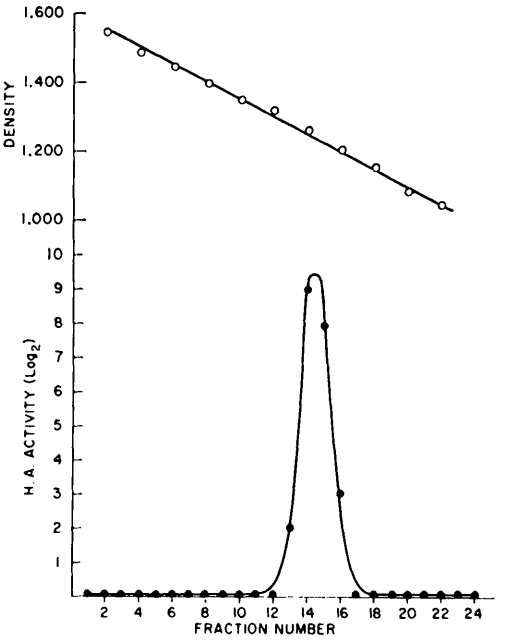

FIG. 3. Equilibrium gradient centrifugation of rubella virus HA in Cs_2SO_4. One ml of antigen was layered over 4.6 ml of a linear 20–60% Cs_2SO_4 gradient and centrifuged for 19 hours in the SW39 rotor at 96,000*g*.

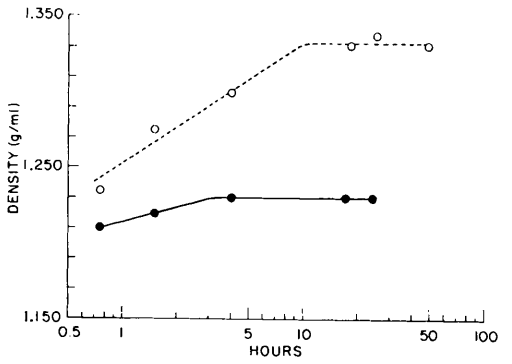

FIG. 4. A comparison of density gradient centrifugation of rubella virus HA in Cs_2SO_4 and CsCl represented by — and ---, respectively. The HA activity was determined on all fractions as in Fig. 3 and the density of each peak was recorded and plotted against centrifugation time.

density 1.23. A statistical analysis of a total of 17 density gradient determinations in CsCl and Cs_2SO_4 indicated that normal curves fit the data well.

Discussion. The density values for rubella virus HA at equilibrium were 1.23gm/ml in Cs_2SO_4 and 1.33 gm/ml in CsCl. Earlier work has shown that the apparent densities of infectious virus and virus antigens vary with the gradient medium used. Thus type A influenza HA has a density of 1.19 in sucrose (12) and 1.25 in CsCl (13); vesicular stomatitis virus has a buoyant density of 1.16 in sucrose and 1.19 to 1.22 in CsCl (14). It has been suggested that the disparities obtained in the different gradient systems result from the effect of concentrated salt solutions on hydration of the virus particle (15). Thus the lower density values of 1.08 gm/ml, 1.18 gm/ml and 1.20 gm/ml previously reported for rubella virus (16–20) may be related to the use of sucrose or citrate gradients. The possibility that the lower values may underestimate the density cannot be ruled out, however, since it is not certain that equilibrium was achieved in these earlier studies. Despite differences in methodology employed in the two studies, our data concerning denisty of infectious virus correlate well with the results reported by Schmidt *et al.* (21). While our preparations were not tested for CF activity, it seems likely from a comparison of the results of these two studies that HA would be localized in the same fractions as the large particle CF antigen.

The sedimentation characteristics of rubella virus HA differed in certain respects from those of other hemagglutinating viruses. In both CsCl and Cs_2SO_4 gradients, HA was localized to a narrow band; the distribution of agglutination activity was very nearly symmetrical about the reaction containing the peak titer. There was no evidence for the heterogeneous array of HA particles of lower density characteristic of influenza A virus strains (13). The rubella virus HA appears to differ from that of measles in its response to treatment with Tween-80 and ether. Measles virus HA was readily separable into 2 components by density gradient centrifugation after Tween-80–ether treatment (22, 23). In contrast, similarly treated rubella virus HA could not be separated from untreated HA by zonal or equilibrium density gradient techniques.

Ultracentrifugation in either CsCl or Cs_2SO_4 effectively concentrated rubella virus HA into a narrow segment of the gradient. The observation that rubella virus preparations grown in BS-C-1 or GMK cells, previously devoid of demonstrable HA activity, showed narrow bands of specific HA after centrifugation in the cesium salt gradients provided a further striking demonstration of this concentration effect.

No differences were observed in the densities of HA produced in several cell cultures by different rubella virus strains or by different virus passage levels. These findings are analogous to those reported earlier for the behavior of respiratory syncytial virus strains in CsCl gradients (24). While tobacco mosaic virus strains of varying densities have been found, the differences noted were extremely small and detectable only with a more precise system than was employed in our experiments (25).

The results of our studies indicate that rubella virus HA and infectivity could not be separated on the basis of either density or sedimentation characteristics. These data provide evidence that rubella virus infectivity and HA are biological manifestations of the same particle.

Summary. The buoyant density of rubella virus HA was determined to be 1.23 in Cs_2SO_4. A density of 1.33 was obtained in CsCl. Rubella virus HA, either Tween-80, ether-treated or untreated, and infectivity could not be separated on sucrose by rate zonal or density gradient techniques. These activities also could not be separated on CsCl and Cs_2SO_4 density gradients. The banding density of rubella virus HA preparations was unaffected by the type of cell culture used for antigen production; neither the passage level nor the strain of virus altered the point at which rubella virus HA banded in CsCl.

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The Effect of Neomycin Sulfate on Pancreatic Lipase Activity (32918)

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Oral neomycin sulfate administration to man frequently leads to steatorrhea (1,2). The mechanism responsible for this association is not yet known. Investigations directed toward elucidating its pathogenesis have logically been centered around digestive and absorptive stages involved in lipid assimilation. There is evidence suggesting that neomycin sulfate may adversely affect both stages (3–5). Recent observations have yielded contrasting results regarding the *in vitro* effect of the antibiotic on lipase activity. Conditions of reported experiments have varied considerably (6,7). Because of the importance of determining what effect neomycin sulfate has on lipase activity, we have