Reports

Density-Gradient Centrifugation with Infectious Ribonucleic Acid of Foot-and-Mouth Disease Virus

Abstract. The sedimentation constant of an infectious component in ribonucleic acid preparations from foot-and-mouth disease virus has been determined by density-gradient centrifugation. A sedimentation constant of 37 Svedberg units (S_f) was obtained. On the assumption that the relation between the molecular weight and the sedimentation constant found by Gierer is applicable to our system as well, a value of 3.1×10^6 was calculated for the molecular weight of the infectious component.

In an earlier study (*1*) we obtained infectious ribonucleic acid (RNA) preparations from the tissues of suckling mice infected in vivo with foot-and-mouth disease virus, type C. The method employed in these investigations was that of Gierer and Schramm (*2*). The isolation of infectious RNA preparations from four animal viruses has been described by others (*3*).

The infectious component in the RNA preparations from foot-and-mouth disease virus differed in some properties from the intact agent.

Recently, Gierer (*4*) was able to calculate from sedimentation and intrinsic viscosity measurements the molecular weight of the RNA from tobacco mosaic virus. It was found to be approximately 2×10^6. This finding encouraged us to determine the sedimentation constant of the active unit in our RNA preparations and to calculate from this value the molecular weight of the active unit.

The virus strain used in this study was type C. The method of Gierer and Schramm (*2*) was employed for the preparation of infectious RNA. The RNA preparations were centrifuged at 35,000 rev/min (95,000*g*) for 5 minutes, and the supernatant was used for determination of the sedimentation constant by density-gradient centrifugation. This centrifugation method was developed because the active unit to be investigated was very labile and made up only a small part of the total RNA content of the preparation. The centrifugations were carried out in a Spinco ultracentrifuge, model L, with a swinging-bucket rotor SW 39. We employed Plexiglas (plastic) cells with a sector-shaped chamber. The chamber had a volume of 1.8 ml and was 3.7 cm high. During the centrifugation the bottom of the chamber was 9.1 cm from the axis. These plastic cells fit in the buckets of the rotor and were manufactured in our workshop. Gradient columns each 6-mm thick were prepared of D_2O—H_2O mixtures containing 90, 76, 61, 44, and 25 percent D_2O, respectively. The *p*H of the mixtures was stabilized with 0.02*M* phosphate buffer. The loaded cells were held at 4°C for 1 hour, then a sample of a RNA preparation was floated on the column. The cells were centrifuged 110 and 130 minutes at 38,000 rev/min (about 112,000*g*) and 35,000 rev/min (about 95,000*g*), and the rates of acceleration and retardation were taken into account. The values of $\omega^2 t$ in the different experiments were in the range from 9.85×10^{10} sec^{-1} to 16.75×10^{10} sec^{-1}. The refrigerator was adjusted so that the rotor had a temperature of 6°C during the whole run. Afterward, the centrifugation samples were removed, in steps, with a special capillary pipette and used for infectivity assay in suckling mice (intracerebral), ultraviolet absorption, and specific-density measurements.

The active unit was detected within a relatively narrow zone. A point in this zone above and below which the same number of infectious units had been found ("mean point") was determined.

The value of the sedimentation constant (s_{20} was calculated from the equation

$$s_{20} = \frac{\sigma - \rho_0^{20}}{\eta_0^{20}} \times R_0 \frac{\int_{R_0}^{R_t} \frac{\eta_R}{(\sigma - \rho_R) \cdot R} \cdot dR}{\omega^2 t}$$

in which σ (1.67 g/ml) represents the specific density of RNA; ρ_0^{20} and η_0^{20} are the specific density and viscosity, respectively, of water at 20°C; ρ_R and η_R are the specific density and viscosity, respectively, of the gradient as a function of the radius; R_0 and R_t are the radii of the "mean point" before and after the run, respectively; ω is the angular velocity; and t is the time.

Functions ρ_R and η_R were determined in a sector-shaped diffusion cell, and the integral was calculated numerically.

In control experiments with hemocyanin from *Helix pomatia* a mean s_{20} of 105 S_f was obtained in an analytical ultracentrifuge, while density-gradient centrifugation furnished a value of 104 S_f, indicating the remarkable accuracy of the method.

The mean s_{20} value of the infectious unit in the RNA preparations obtained from foot-and-mouth disease virus was 37 S_f.

If one assumes that the infectious unit in our RNA preparations has the same structure as the RNA of tobacco mosaic virus, the relationship between the sedimentation constant and the molecular weight found by Gierer (*4*) may be used for calculation of the molecular weight of the infectious unit. A value of 3.1×10^6 was obtained.

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References

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

Instrumental Conditioning of Lemon Sharks

Abstract. Two sharks were trained to feed at a target which, when pressed, caused a submerged bell to ring. Later they were trained to press the target for remotely placed food. They retained this conditioned response after a 10-week period of inactivity.

Captive sharks, like other fishes, quickly learn to go to the place where they are usually fed. Experiments were conducted to determine the extent to which they could be conditioned to more complex situations (*1*).

The sharks used were a male and female lemon shark, *Negaprion brevirostris* (Poey), each about 3 m long. They had been in captivity over 4 months and were healthy and active. They were