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

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

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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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

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

Table 1. Weekly summary of number of bell rings by five sharks.

Week No.	Total for two lemon sharks			Total for three nurse sharks			Place of food†	Av. water temp. (°C)
	Rang bell, got food	Rang bell, missed food	Food "stolen"*	Rang bell, got food	Rang bell, missed food	Food "stolen"*		
1	70	11	0	5	0	(3)	0	29
2	99	10	0	8	0	(4)	0	28
3	88	25	0	15	0	(2)	0	27
4	101	14	0	11	1	(21)	0	25
5	80	23	0	9	4	(36)	0	23
6	70	26	0	31	5	(9)	0	22
Total	508	109	0	79	10	(75)		
7	27	57	2	15	13	2	66	23
8	56	65	12	7	12	1	96	23
9	56	94	39	5	8	8	126	23
10	35	73	31	0	2	13	156	24
11	27	32	15	0	2	6	216	21
12	18	24	6	0	1	12	216	21
13	4	9	3	0	0	1	246	17
14	0	0	0	0	0	0	246	17
Total	223	354	108	27	38	43		
Grand total	731	463	108	106	48	118		

* Food "stolen" after another shark rang bell. Figures in parentheses represent food sucked off target without a ring. † Distance (in centimeters) from center of target.

housed in a 12- by 18-m pen, adjacent to the laboratory dock, with three nurse sharks, *Ginglymostoma cirratum* (Bonnaterre), of about the same size (males).

During a 6-weeks' training period the sharks were fed five times a week, Monday through Friday, at approximately 3:15 P.M. At this time a target was lowered into the water, and it was removed at the end of the feeding period. (It was never put into the water at any other time.) The target was made of a piece of plywood 41 cm square and painted white. When the target was pressed it caused a submerged bell to ring.

For the first 2 days of the training period pieces of food were thrown to the sharks near the target. Gradually the food was thrown closer to the target. On the third day, and for the rest of the 6 weeks, the food was tied to the center of the target on a short, weak string. In order for the lemon sharks to feed, they were forced to press their snouts against the target. Food was on the target when it was first lowered into the water, and as a shark removed the food, another piece was supplied.

At the beginning of the seventh week, an empty target was lowered into the pen at the regular feeding time. When a shark pressed the target hard enough to ring the bell, a piece of reward food attached to a string was dropped into the water. The shark was given 10 seconds to get this food, and if he did not succeed, the food was then pulled out of the water. Each week the food was dropped farther away from the target.

The results of these feedings are summarized in Table 1. During the training period the lemon sharks rang the bell and successfully obtained food at the target 508 times; they rang the bell but missed the food 109 times. In contrast to the lemon sharks, who, on approaching the target, only slowed down in an effort to take food from it, the nurse sharks would approach the target from below, hover beside it, and move their oral barbels along the target's surface. When they had located the food, the nurse sharks often were able to suck the food off the target without pressing the target hard enough to ring the bell. This they did a total of 75 times. They rang the bell and took food at the target 79 times and missed the food after a ring only ten times. The latter score also reflects their ability to hover before the target.

The first time the sharks were confronted with an empty target, the male lemon shark approached it in less than half a minute after it was lowered into the water. When he reached the target he slowed down and merely brushed the target, without pushing it hard enough to ring the bell. After repeating this maneuver nine times, he finally pressed the target sufficiently hard to ring the bell, and food was immediately dropped into the water. The male quickly learned to press the target for reward food, and by the end of the week both the male and the female lemon sharks were successfully conditioned to pressing the empty target and returning for food. The individual scores for the male and the female were closely alike and will be analyzed elsewhere.

The data accumulated in the weeks of testing with the empty target are shown in the lower portion of Table 1. The nurse sharks did not appear to make a strong association of the target with food. At first they came to the target, but then they started to spend more time hovering under the food and frequently took the food dropped after a lemon shark had rung the bell. The lemon sharks also took food from each other. When one shark rang the bell and another shark took the food we scored this as a "steal" for the shark that got the food.

When the water temperature dropped below 24°C the sharks fed less, and by mid-December they lost interest in food offered to them in any manner. Until this time, however, the lemon sharks rang the bell and got the remotely placed food 223 times; they rang the bell but missed the food 354 times; they "stole" food 108 times. The nurse sharks rang and got food 27 times, rang but missed the food 38 times, and "stole" food from the lemon sharks 43 times. The presence of nurse sharks hovering around the feeding place led to frequent collisions among the sharks.

There was a strong tendency for the male lemon shark to approach the target first at each feeding period. Scores were kept of the chronological order of approach during 53 feeding periods, during which time the lemon sharks rang the bell 1117 times. The male rang first 50 times and the female, three times. Of the first three rings made during each of these feeding tests, 134 were made by the male and only 25 by the female. As the female was placed in the pen several weeks before the male, prior residence was not a contributing factor in what may be social dominance of the male. "Fighting" among the sharks has never been observed. We have no evidence yet in explanation of the fact that the female refrains from pressing the target until the initial hunger of the male apparently is satisfied.

The sharks retained these conditioned responses through the midwinter cold spell. When the temperature rose to over 20°C again, the sharks readily pressed the target when it was presented to them, even though they had not seen it for a period of 10 weeks.

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Notes

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