MEASUREMENT OF PELLET VOLUME IN THE ANALYTICAL ULTRACENTRIFUGE*

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The analytical ultracentrifuge[1] is usually employed either for study of sedimentation equilibrium or for measurement of sedimentation velocity of suspended materials. In this paper is described a third procedure which has given new information on some animal viruses, namely, direct measurement of the volumes of the pellets sedimented. Although measurement of the amount of sedimented material accumulated in the pellet after low speed centrifuging is common practice, studies of pellet volumes in the ultracentrifuge during operation do not seem to have been made. Such studies are potentially capable of yielding information not only about the quantity of material sedimented, but, if combined with other data, may give evidence of the degree of purity and nature of the material as well. If pure materials are used, some of their physical properties may be revealed, as indicated in the present paper, by pellet volume measurements at various rotor speeds and after various times of exposure to the centrifugal force.

By the procedure to be described, the volume of sediment collected at the bottom of a transparent ultracentrifuge cell is measured from photographs taken while the machine runs. The accumulation of deposit can be observed directly, and, if the pellet thus formed is compressed by further operation of the centrifuge or by increase in rotor speed, this too can be followed. The degree of accuracy to be expected from such measurements will be demonstrated in connection with an experiment showing some properties of purified swine influenza virus.

THE ROTOR CELL

In Fig. 1 there is shown the duralumin rotor from the air-driven vacuum type analytical ultracentrifuge used in this work. Its radius to the center of the cell holes is 6.5 cm. One hole contains the dummy cell or balance weight; the other is empty, and lying near it in the photograph is the specially designed cell made of transparent lucite (methyl methacrylate resin), the details of which are shown in Fig. 2. Through the body of the cell, a small hole is drilled and then finished with a #1 taper-pin reamer. The bottom is permanently closed by cementing on a small sliver of lucite with the solvent, ethylene dichloride. At the top, a stainless steel cover, shaped to give maximum height of hole, is held in place over a small piece of thin rubber gasket by two fillister head

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0–80 brass screws. This seals the hole which contains the suspension under study. Two views are shown in Fig. 2. The one at the right is a view from an imaginary cutting plane indicated by A–A in the center view. On the left end of the center view, and in

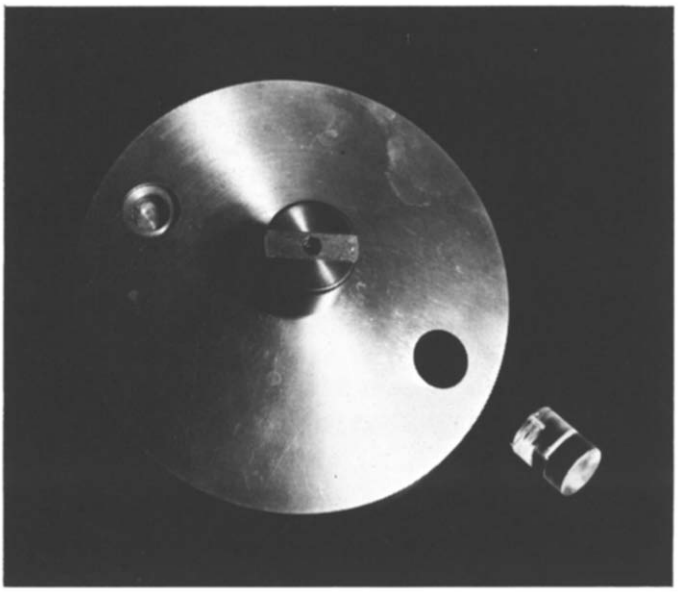

Fig. 1. Duralumin rotor of the air-driven vacuum type ultracentrifuge. At the left is the dummy or balance cell in place, and opposite it is the lucite cell for pellet volume measurements.

the left round projection is shown the pair of "D" shaped black bakelite pieces held on by brass screws*, which act as masks limiting a beam of parallel light passing endwise through the cell to a band slightly narrower than the hole containing the suspension under study.

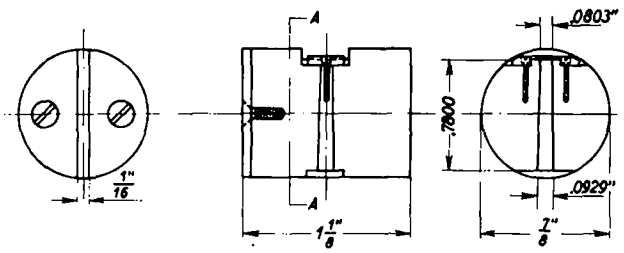

Fig. 2. Lucite rotor cell showing "D" shaped masks at the left. At the center and right are side and cross-section views showing the tapered hole containing the suspension to be centrifuged.

OPTICAL ARRANGEMENT AND PHOTOGRAPHIC PROCEDURE

Light from a capillary mercury arc** is used with all lenses, mirrors, etc., arranged according to the method of SVEDBERG[1] for light absorption photographs of sedimenting

* Aluminum screws would probably be better at very high rotor speeds.
** Lamp type H_4 Westinghouse Elec. & Mfg. Co.

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boundaries. No light filters are necessary. For highest photographic accuracy, fine grain film is desirable. At present, however, there is being employed the coarser but much faster Eastman Super Panchro Press type B film with which exposures of 1 to 5 seconds are sufficient to produce properly exposed films. Fig. 3 shows a series of pictures made of the pellet from a 20 mg/ml suspension of swine influenza virus as it is compressed gradually to constant volume in the rotor cell. Each picture is the result of a pair of

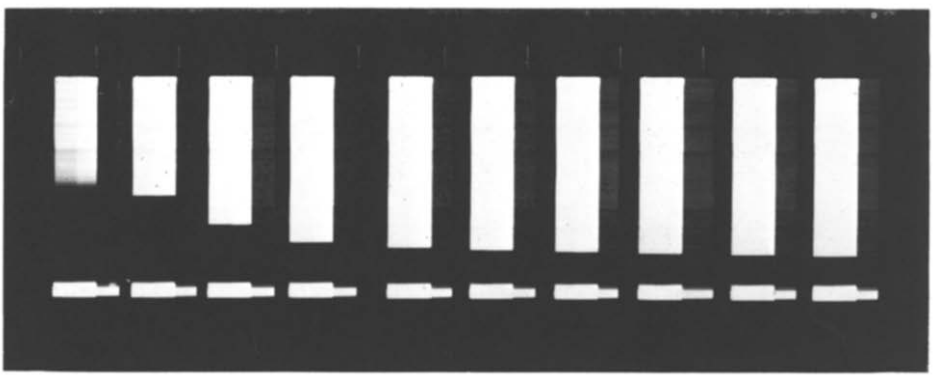

Fig. 3. A typical series of pictures showing, from left to right, the compression with time of a swine influenza virus pellet at constant rotor speed. Overlapping exposures aid in measuring pellet thickness.

exposures of different duration taken so as to given a choice of three degrees of blackness from which to choose at the time of measurement. Light passes readily through the supernatant fluid above and even more readily through the clear lucite below the pellet. The black band across each picture, decreasing in width from left to right, is formed by the pellet. Measurement of the width of this band on the negative with a travelling microscope gives pellet height. It will be seen that whereas the top edge of the black band is exceedingly sharp the bottom edge is not. In order to get greater accuracy with smaller pellets, use has sometimes been made of a point of reference other than the cell bottom for measurements. This has been done by drilling a small hole in the balance weight (Fig. 1). Through it passes a sharp point of light which draws a suitable reference line on the photographs.

ILLUSTRATIVE EXPERIMENT

In order to clarify certain details of procedure, the following experiment will be described. The cell was filled (0.075 ml) with an 18.4 mg/ml suspension of purified swine influenza virus[2] in mammalian RINGER solution and the centrifuge brought to a constant speed of 7500 rpm. Virus in this concentration is turbid in suspension and is easy to see by watching the scanning image on the ground glass of the ultracentrifuge camera. When the supernatant fluid became clear, pictures like those of Fig. 3 were taken, yielding the pellet volumes plotted against time in the curve of the squares of Fig. 4. These volumes were obtained from measurement of the virus pellet pictures together with calibration pictures. Calibration of the cell-camera arrangement was made with weighed amounts of mercury in the range of pellet volumes under investigation. Each amount was photographed at the various rotor speeds to be used in the experiment.

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If the remaining space in the cell above the mercury is filled with water, the mercury interface shows up much more clearly in the picture than it does if no water is used.

Pellet volumes at the 7500-rpm-speed decreased rapidly at first then more slowly, seeming to attain a minimum in a little over six hours after all virus was sedimented. Another cell full of the same virus suspension was then spun at 30 000 rpm, and the

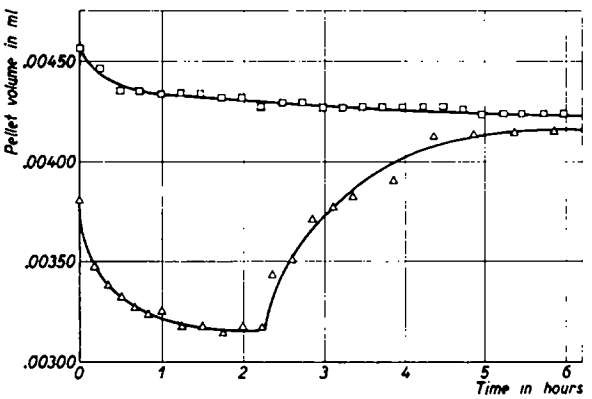

Fig. 4. The volume of a pellet sedimented from a suspension of purified swine influenza virus. Time is measured from T = 0 when visual observation showed no more virus in the supernatant fluid. At the top (squares) the rotor speed was 7500 rpm. At the bottom (triangles) is the same amount of virus held at 30 000 rpm for 2½ hours after which the speed was reduced quickly to 7500 rpm.

volumes obtained are shown in the decreasing part of the curve of triangles in Fig. 4. The minimum pellet volume was reached in 2¼ hours, after which the rotor speed was decreased quickly to 7500 rpm, the speed of the previous run. The increasing pellet volumes were recorded to a total of six hours, during which time the pellet volume approached asymptotically to within 1½% of the value attained in the upper curve. It is necessary, of course, to maintain constant rotor speed for several hours for these runs, and, to do this, an electronic governor was built which varies the drive air pressure according to the needs of the machine and independently of variations in supply pressure.

DISCUSSION AND CONCLUSIONS

From the curves of Fig. 4 and the photographs of Fig. 3, one would expect reliable volume measurements in the region of 0.004 ml to ± 1%. This is probably a conservative estimate, as many repeat runs at this and lower pellet volumes have yielded results well inside this value. About 75 series have been run thus far.

Some consequences of this experiment other than technical ones serve to illustrate the use of the method. The virus forms a pellet which compresses, in a constant centrifugal field, slowly with time, approaching a minimum value only after several hours at 7500 rpm. At greater speed, 30000 rpm, the pellet volume approaches more quickly a lower value. This may in itself suggest volume elasticity, but elastic behaviour is definitely demonstrated when, on reduction of the accelerating field, the pellet expands almost to its previous value. Further work is being done to study this behaviour of the sedimented virus pellet in the light of independent data on hydrated density of the virus particles.

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SUMMARY

The analytical ultracentrifuge is ordinarily used to observe sedimentation equilibrium or to measure sedimentation velocity. This paper describes its use for measuring the volume of sedimented material. By means of a transparent plastic rotor cell this volume, of the order of 0.004 ml, can be measured to about ± 1%. When purified influenza virus was sedimented in this cell, the pellet volume was related to rotor speed in an elastic manner. The nature of the adjustment of pellet volume to rotor speed can be followed continuously by photographic means.

RÉSUMÉ

L'ultracentrifugeuse analytique est employée en général pour observer l'équilibre de sédimentation et pour mesurer la vitesse de sedimentation. Dans ce mémoire nous décrivons son emploi pour la mesure du volume du matériel sédimenté. Si l'on emploie comme rotor, une cellule plastique transparente l'on peut mesurer ce volume qui est de l'ordre de 0.004 ml avec une précision d'environ ± 1%. Si du virus purifié de grippe est sédimenté dans cette cellule le volume du dépôt est relié à la vitesse du rotor de façon élastique. L'on peut suivre de façon continue par photographie l'adaptation du volume du dépôt à la vitesse du rotor.

ZUSAMMENFASSUNG

Die analytische Ultrazentrifuge wird im Allgemeinen zur Beobachtung des Sedimentationsgleichgewichtes und zur Messung der Sedimentationsgeschwindigkeit verwendet. Hier wird ihre Anwendung zur Messung des Volumens der sedimentierten Substanz beschrieben. Unter Verwendung einer durchsichtigen plastischen Zelle als Rotor kann dieses Volumen von der Grössenordnung 0.004 ml mit einer Genauigkeit von ungefähr ± 1% gemessen werden. Wurde gereinigter Grippe-Virus in dieser Zelle sedimentiert so war das Volumen des Depots elastisch von der Rotorgeschwindigkeit abhängig. Die Anpassung dieses Volumens an die Rotorgeschwindigkeit kann kontinuierlich durch Photographie verfolgt werden.

REFERENCES

[1] THE SVEDBERG AND K. O. PEDERSEN, *The Ultracentrifuge*, Oxford Press, 1940.
[2] A. R. TAYLOR, D. G. SHARP, I. W. MCLEAN, JR., DOROTHY BEARD, AND J. W. BEARD, *J. Immunol.*, 48 (1944) 361.

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