Reports

Detection and Titration of Asian Influenza A Virus by HeLa Cell and Monkey Kidney Cell Cultures

Vogel and Shelokov (*1*) have described a method whereby the presence of influenza virus may be detected with relative ease in monkey kidney cell monolayer cultures. Influenza virus was first adsorbed on monkey kidney monolayers, and later guinea pig erythrocytes were agglutinated on the infected cell sheet. In this manner visual evidence of infection was elicited. Our studies have confirmed these results, and the method has been extended to include the quantitative titration of influenza virus and the use of HeLa cell cultures for both detection and titration. A comparison of this method of titration with the classical hemagglutination (HA) titration suggests that the new method is many times more sensitive than the hemagglutination tests.

Five-day-old primary cultures of monkey kidney cells prepared by the method of Youngner (*2*) and grown in medium M202 (*3*) with 2 percent rabbit serum were used throughout the experiments.

Eight-day-old monolayer cultures of cell strain HeLa (Gey) completely adapted to growth on rabbit serum supplemented medium were used in these experiments in either T-9 flasks or screw-cap tubes. The HeLa cells were grown in a routine medium consisting of 15 percent rabbit serum and Scherer's maintenance solution MS (*4*).

The strain of influenza A used had been isolated locally in eggs and was identified as Asian type. It had been designated A/NS/45/57, and the current stock after five amniotic passages in 10-day-old embryonated eggs had a hemagglutination titer by the method of Salk (*5*) of 80 HA units per milliliter. This HA titer was checked frequently throughout the experimental period so that a comparison could be made of the sensitivity of titration methods. All dilutions of A/NS/45/57 were made in the same diluent, Scherer's MS with 5 percent rabbit serum, whether the cells to be infected were monkey kidney or HeLa.

Monkey kidney or HeLa cell monolayers were infected with serial half $\log_{10}$ dilutions of stock (80 HA units per milliliter) influenza virus A/NS/45/57. Three screw-cap tubes or T-9 flask cultures were infected at each dilution of virus. The infected cultures and uninfected controls were then incubated at 37°C for 48 hours.

After incubation the cultures were washed twice with phosphate-buffered saline (*6*), and 1.0 ml of a 1 percent suspension of fresh chicken erythrocytes in phosphate-buffered saline was pipetted into the culture flask. This suspension was allowed to remain over the cell sheet for 5 minutes, during which time the erythrocytes sedimented onto the monolayer. After 5 minutes the cultures were again washed gently twice in phosphate-buffered saline and stained.

The washed cell sheets, either HeLa or monkey kidney cells, were first dried and fixed for 1 minute in purified methanol and then stained for 5 minutes with 0.15 percent Leishman's stain in acetone-free methanol. The preparation was then washed twice with distilled water and allowed to stand for 10 minutes before observations were made.

Preparations were examined at a magnification of 60 with a microscope, the eyepiece of which had been adapted so that the field was square and bisected by grid lines into four smaller squares. The clumps of agglutinated erythrocytes had stained pale pink and contrasted with the pale blue cell background.

During the counting procedure all cultures were selected and numbered at random, and the operator was unaware of dilution values. Figure 1 shows the results obtained with both HeLa and monkey kidney cell cultures. In this figure each point is the average of six field counts and two separate experiments (three field counts at each dilution per experiment). The results show that a straight line gives a reasonable fit to the data plotted as the number of clumps versus dilution of virus. It can also be appreciated from Fig. 1 that the amount of virus which can be detected by the titration method corresponds to roughly 0.08 HA units of virus.

In order to test the specificity of this clumping reaction, use was made of various types of influenza antisera. The antisera, in threefold dilution, were placed over the infected and control HeLa cell monolayer cultures for 1 hour before the cultures were processed as described above for clump counting. Neither B-Lee, FM1 nor A-Can (A-prime strain) antisera reduced the ability of infected cell sheets to agglutinate and clump erythrocytes. However, a hamster antiserum specific for Asian influenza A virus prevented clumping up to a dilution of 1:90.

During a recent influenza epidemic sputum specimens were collected from suspected cases and processed by classical embryonated egg isolation methods for the isolation of influenza virus. Some of the sputum specimens from which influenza virus type A (Asian) was isolated were used to infect monkey kidney and HeLa cell monolayers. One milliliter of these specimens, in 3 percent tryptose phosphate broth, was placed on the cell sheet and incubated at 37°C for 1 hour. The sputum specimen was then removed and replaced with 5 percent rabbit serum in Scherer's MS. Cultures thus treated were then incubated again for 72 hours and processed for clump counting as indicated above.

With HeLa cells, six out of seven egg-positive specimens were also considered positive by the adsorption hemagglutination method. A specimen was considered positive if at least five well defined clumps of chicken erythrocytes could be counted on a routine screw-cap tube monolayer culture. With monkey kidney cells, it was considered that all five specimens out of five processed were positive.

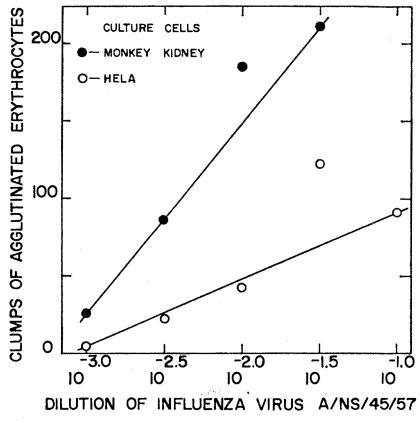

Fig. 1. Titration of influenza virus A/NS/45/57 by clump counting.

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. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

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)].

This method of detecting influenza virus may be of use where tissue-culture methods are deemed more suitable than isolation by means of the embryonated egg. However, we wish to point out that the egg isolation method has been found by us to be many times more sensitive. The stock virus A/NS/45/57, containing 80 HA units per milliliter and with an end-point titration by the adsorption-hemagglutination method of about $10^{-3.1}$, has an egg infective dose 5 percent of close to 10^{-8}.

K. R. Rozee
G. L. Williams
C. E. van Rooyen
Department of Bacteriology, Dalhousie University, Halifax, Nova Scotia

References and Notes

1. J. Vogel and A. Shelokov, *Science* 126, 358 (1957).
2. J. S. Youngner, *Proc. Soc. Exptl. Biol. Med.* 85, 202 (1954).
3. Medium M202 is a variation of medium 199 [J. G. Morgan, H. J. Morton, R. C. Parker, *ibid.* 73, 1 (1950)].
4. W. F. Scherer, *Am. J. Pathol.* 29, 113 (1953).
5. J. E. Salk, *J. Immunol.* 49, 87 (1944).
6. R. Dulbecco and M. Vogt, *J. Exptl. Med.* 99, 167 (1954).

16 April 1958

Instantaneous Linear Velocity of Flow in Pulmonary Artery Measured by a Catheter Tip Method

Abstract. Measurements of the instantaneous linear velocity of blood flow in the pulmonary artery of the human have not been reported previously. This report describes a catheter tip method for making such measurements.

A double-lumen catheter was designed (*1*) with one opening in the tip and the other 4 mm from the tip on the side of the catheter. When the catheter is positioned in the pulmonary artery, the pressure recorded from the tip is less than that from the side opening, because of the "drag" of the blood flowing past the tip. Accordingly, in this method, the catheter is used as a Pitot tube facing away from the approaching stream rather than into it as in the usual application of the Pitot tube. With the use of standard pressure transducers of equal sensitivity it is possible to record the difference in pressure which exists between the tip and side openings. The pressure difference is related to the velocity of the stream at the tip of the catheter by the equation

$$v = c(2g\Delta p)^{1/2}$$

where v is the velocity, g the gravitational constant, Δp the recorded pressure difference, and c a constant, all expressed in consistent units. The value of c, 0.3 for forward flow, was determined by repeated, reproducible measurements of Δp with the catheter in a long straight tube through which water was forced at different, known rates of flow. The catheter in this, and in other studies made with pulsatile flow, tended to seek the axis of the tube and showed a minimum of vibratory motion or "whip." Nor was significant whip noted in the measurements in the pulmonary artery reported here. For retrograde flow, a value of 0.8 was assumed for c.

In Fig 1 are shown the almost superimposed pressure tracings from the two lumina of the catheter, obtained in the pulmonary artery of an adult. The zero point of the pressure curve from the tip was arbitrarily displaced slightly above that of the pressure curve from the side opening so that the pressure tracing from the tip lies below that from the side opening only during periods of rapid forward flow, when the tip pressure was so reduced by the "drag" as to offset the displacement of the zero point. The pressures were recorded at two points, one close to the pulmonic valve, the other in a major branch of the right pulmonary artery, well distal to the valve. Shown below the pressure tracings in Fig. 1 are the corresponding curves of the difference in pressure between the tip and side openings.

The pressure tracings recorded from the two sites in the pulmonary artery are technically satisfactory. Aside from slight differences in the systolic and diastolic pressures, they differ chiefly in that the dicrotic notch and the succeeding rise in pressure occur later in the tracings from the distal position. The differential pressure curves differ in several respects. Close to the valve, there is a rapid increase in the velocity of ejection immediately following the onset of systole. The maximum velocity (29 cm/sec) occurs just prior to the pressure maximum; the velocity then decreases rapidly to zero. The entire period of forward flow lasts 0.2 sec and ends just before the appearance of the dicrotic notch. There follows a period of backward flow lasting just over 0.1 sec and ending with the completion of the dicrotic notch. It is succeeded by a short period of lesser forward flow, which, in turn, is followed by two or three small oscillations before the start of the next ejection. The duration and timing of the backward flow suggest that it is associated with the retrograde movement and closure of the semilunar cusps.

These curves differ from the only other published curves of instantaneous velocity in the pulmonary artery (*2*) in that back-flow is a significant feature of the curves presented here, whereas the curves of Baxter and Pearce, obtained in dogs with an implanted Pitot tube in the usual orientation, showed no back-flow.

In the pressure tracings from the distal point in the right pulmonary artery, the dicrotic notch and the postnotch rise in pressure are seen to occur later than in the tracings taken close to the valve. In fact, with some of the beats, the postnotch rise merges with the systolic rise of the next beat. That this rise is not due to atrial systole is shown by the lack of any constant temporal relationship between the rise in pressure and the appearance of the P-wave. Examination of the differential pressure curve shows that there is a significant forward flow associated with the post notch pressure rise, suggesting that this flow is due to a surge of blood rebounding from the valve and the arterial segment immediately distal to the valve following the retrograde flow noted above and the sudden closure of the valve. The systolic flow rises to a maximum (22 cm/sec) in two steps and returns quickly to minimal flow, the whole ejection lasting just over 0.3 sec. No sign of more than momentary and insignificant retrograde flow is noted at this point in the artery.

The method is not technically difficult. A properly modified double-lumen catheter is required in which the dynamic characteristics of the two lumina are identical. Pressure transducers of equal sensitivity connected to a recording apparatus that can produce a signal pro-

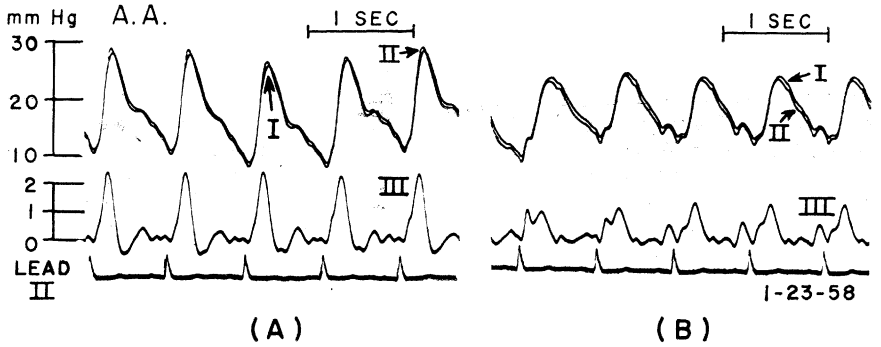

Fig. 1. (*A*) Catheter just distal to pulmonic valve. Curves I and II, pressures from tip and side openings, respectively. Curve III, differential pressure (note different scales). (*B*) Catheter in branch of right pulmonary artery. Curves same as in *A*.