phatase in the substance of the muscle fibers is increased in atrophied fibers. Where muscle fibers are being replaced by connective tissue, the invading cells show a high level of acid phosphatase activity.

Alkaline phosphatase in normal human muscle is limited to the capillaries and the intima of larger vessels. In dystrophies, the enzyme occasionally appears in the atrophying muscle fibers and in the very fine connective tissue fibers found surrounding them.

The increase of phosphatase activity (including 5-nucleotidase) in the connective tissue of muscles in some types of dystrophy suggests that phosphatases may play an important part in the destruction of muscle fibers associated with these diseases. Even if they are not directly concerned with the breakdown of muscle substance, they may cause functional failure through excessive breakdown of high-energy phosphate compounds. A similar process may be involved in aging (*5*).

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6 May 1957

Resistance to Desiccation in Intertidal Barnacles

The resistance to desiccation on exposure to air is recognized to be an important factor in the zonation of intertidal animals, and it is achieved in a variety of ways by different species. In spite of the work of Monterosso (*1*) on *Chthamalus stellatus* var. *depressus*, it is frequently stated that barnacles that live relatively high up on the shore achieve their resistance to desiccation by enclosing a small quantity of water in the mantle cavity as the tide recedes. This is not so.

Although morphologically they are very similar, littoral and sublittoral barnacles behave very differently when they are removed from water. In general, the intertidal species such as *Chthamalus stellatus, C. fragilis, C. dalli, Balanus balanoides* and *B. glandula* show what may be termed a "controlled" behavior pattern that leads to adjustment to the new environment. On the other hand, sublittoral species such as *Balanus crenatus* or *B. balanus* struggle in an erratic fashion and soon become desiccated.

The most detailed observations have been made on the intertidal *B. balanoides*. When these are first removed from water, the cirri, in a collapsed state, are partially extruded several times, from the mantle cavity with the expulsion of water. Such movements are repeated at intervals during the first few minutes of exposure to air. Very soon this activity decreases. The valves, still far from being fully retracted, then come together in such a way that the underlying, uncalcified folds of the operculum form a small, diamond-shaped, micropylarlike opening. There is, therefore, direct access for air to the mantle cavity.

Subsequent macroscopic movements of the valves may be accompanied by further expulsion of small droplets of water; such droplets are often found on the valves of shore animals during intertidal periods. In between such macroscopic movements, and subsequent to them, the micropylar orifice is open and, from time to time, appears to be subject to regular pulsation—some 40 pulsations per minute. After several hours the valves may completely close from time to time; this is accompanied by reformation of the micropylar opening, whose size decreases with time. Over a period of 24 hours the valves are not fully retracted within the shell; complete retraction takes place only when the valves are touched (this is doubtless a defense mechanism against predators, which usually attack the animal at the opercular valves).

Although extremes of desiccation attributable to high temperature or long exposure to air may result in complete closure, the evidence indicates that, during an intertidal period of several hours, the barnacles are utilizing atmospheric oxygen in their respiratory activity. That this is the case is also supported by the fact that we have found that, after the barnacles have been exposed to air for several hours, there is no significant accumulation of lactic acid and no accumulated oxygen debt such as might be expected if anaerobic processes were involved.

When animals, after exposure to air for some time, are reimmersed, the operculum is quickly opened (in *B. balanoides*, this occurs within from 10 to 15 seconds), and bubbles of gas escape from the mantle cavity. This precedes extrusion of the cirri, amongst which gas bubbles are often entangled, and resumption of normal cirral activity (*2*).

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Adsorption-Hemagglutination Test for Influenza Virus in Monkey Kidney Tissue Culture

In the course of investigations of influenza viruses in monkey kidney tissue culture, it was observed that addition of erythrocytes directly to tubes with viral cytopathic effect resulted in adsorption of erythrocytes onto the monocellular epithelial sheet. Further studies indicated that this is a specific phenomenon dependent on the hemagglutinating property of the virus. Besides contributing to clearer understanding of the mechanism of hemagglutination, the reaction may be of practical importance in diagnostic work. The purpose of this preliminary report is to describe our technique because of its possible value to rapid diagnosis of suspected cases of influenza. This is pertinent to studies of the influenza epidemic which has already involved major portions of the Far East.

Monkey kidney cells in monolayer sheets were prepared by a modified Youngner technique (*1*), grown in a medium recommended by Melnick (*2*), and maintained in a nutrient mixture (*3*) of 75 per cent Earle's balanced salt solution, 24 percent bovine serum ultrafiltrate, and 1 percent heated horse serum with appropriate antibiotics. Calf serum was not used in the maintenance medium because of its possible anti-viral effect. Most of the experiments employed an influenza virus strain recently isolated in this laboratory directly in tissue culture; this strain was provisionally designated A/Md/1542/57 and was found to be closely related to A/Md/1/55.

Our current technique is as follows. Supernatant fluids are removed from infected and control monkey kidney tubes. Two-tenths of a milliliter of 0.4-percent suspension of washed, citrated guinea pig erythrocytes are added to each tube (results with influenza A were not as consistent using chicken erythrocytes). The tubes are placed in horizontal position for about 1 minute, and the initial reading is made by microscopic examination under low power for clumping of erythrocytes in the fluid and visible adsorption-hemagglutination. With experience, this examination is often sufficient to determine whether the test is positive or negative. Because the settling of erythrocytes onto the monolayer sheet during standing, especially if it exceeds a period of a few minutes, may resemble a

positive reaction, it is generally advisable to wash the tubes with 1 ml of Earle's balanced salt solution or maintenance medium after the preliminary reading and replace the discarded fluid. This removes most of the unadsorbed erythrocytes, leaving a clearly visible monkey kidney cell sheet studded with clumps of agglutinated red blood cells adhering to the tissue in an easily recognizable characteristic pattern.

Figure 1*a* shows such a positive reaction 4 days after inoculation of the tube with a strain of influenza A virus. For comparison, photographs of a similarly treated uninoculated tube of the same age (Fig. 1*b*) and an untreated but virus-inoculated tube (Fig. 1*c*) are also included. Only a few floating erythrocytes against the background of healthy cells are seen in the normal control, while the infected tube shows typical cytopathic effect observed prior to addition of red blood cells.

Inasmuch as the readability of the adsorption-hemagglutination test depends on the presence of an adequate area of monolayer cell sheet, the test should be performed before the cell culture is destroyed either by far advanced cytopathic effect or by degenerative changes caused by aging. At first monkey kidney tubes were always tested for adsorption-hemagglutination after specific cellular degeneration was observed, but subsequently it was found that in many cases a positive test could be obtained prior to the onset of the cytopathic effect.

The specific nature of this phenomenon is indicated by the following facts. (i) Although several batches of monkey kidney cell cultures of various ages and stages of nonspecific degeneration were tested, a positive test was never observed in the absence of hemagglutinating viruses. (ii) Cytopathogenic strains of influenza A virus, originally isolated in tissue culture or in eggs and identified by the conventional hemagglutination and hemagglutination inhibition techniques, never failed to give a positive adsorption-hemagglutination test. (iii) Finally, the adsorption-hemagglutination reaction can be completely inhibited by the addition of specific immune rooster serum known to inhibit hemagglutination by the same virus isolate, but not by antiserum prepared against other types of influenza or certain unrelated viruses, such as Coxsackie A and B, ECHO, and polioviruses. Incomplete studies suggest that the reaction is inhibited only by serums against closely related type A strains.

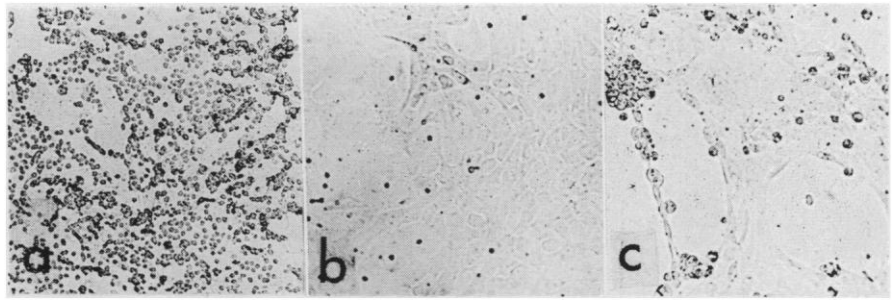

Fig. 1 (*a*) Positive adsorption-hemagglutination test with guinea pig erythrocytes in monkey kidney cell culture 4 days after inoculation with influenza A virus (×90). (*b*) Similarly treated normal monkey kidney cell culture (×90). (*c*) Typical influenza virus effect in monkey kidney cell culture of the same age, prior to addition of erythrocytes (×90).

This adsorption-hemagglutination test may be a useful procedure in rapid diagnosis of suspected cases of influenza. The use of this test is particularly attractive because it depends on direct visualization. We have observed positive adsorption-hemagglutination tests in monkey kidney cultures inoculated with seven strains of influenza A. Two recent isolates, A/Md/503/57 and A/DC/1195/57, gave positive tests 3 to 5 days after direct inoculation with 0.2 ml of throat swab suspensions. An egg-isolated strain of Far Eastern influenza A virus (A/Jap/305/57) was positive after each of the four passages in monkey kidney tissue culture, and another Far Eastern strain (A/Formosa/313/57) was positive upon passage of the egg material in tissue culture (*4*).

The adsorption-hemagglutination reaction with other hemagglutinating viruses is now being studied in tissue culture systems.

Note added in proof: Since submission of this manuscript, we were able, possibly because of an important change in the technique of the test, to isolate several virus strains from cases of suspected Far Eastern influenza in the United States. Standard monkey kidney cell cultures were changed to Eagle's medium *without any serum,* inoculated with field specimens, and placed in a rotating drum. Twenty-four to 48 hours later guinea pig erythrocytes were added, and the tubes were examined for adsorption-hemagglutination. A homogenized suspension of kidney cells, erythrocytes, and fluid from the positive tubes was inoculated into fresh cultures. This passage was used for identification by inhibition of the reaction with type specific antiserum. Twenty-three specimens from a July 1957 outbreak were tested by this procedure and the conventional embryonated egg technique. Of these, the same eight specimens were found to be positive by both methods. So far, seven of the isolates were shown to be closely related to strain A/Jap/305/57. We were able to detect most of the positive specimens at the time of initial addition of erythrocytes to cell cultures, well in advance of the earliest egg isolation results.

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