

Factors Affecting Growth of Mammalian Viruses in Vitro

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Multiplication of viruses, rickettsiae and some other micro-organisms is intimately associated with living tissues. This association presents certain difficulties in the determination of growth requirements of these agents. On the other hand, it is possible to investigate host-parasite relationships under extremely favorable conditions.

A number of viruses and rickettsiae have been propagated experimentally in laboratory animals, embryonated eggs and in excised tissues. The following discussion will be limited to a few factors which influence growth of these agents *in vitro*—i.e., in the test tube.

The methods suitable for the cultivation of viruses and rickettsiae are three: 1) The Carrel technique, using fragments of tissues embedded in a drop of clotted plasma. This procedure was introduced in 1927 (1). 2) In the following year, Maitland and Maitland (2) achieved the same result using tissue fragments bathed in Tyrode's solution containing a little normal serum. This is not a true tissue culture procedure since little or no proliferation of tissues occurs. 3) In 1939, Gey and Bang (3) and, in 1940, Enders and associates (4) introduced the roller tube method for cultivation of viruses. This procedure permits cultivation of an infectious agent without interruption for several weeks.

Time. The growth curve of a virus cultivated *in vitro* usually can be divided into lag, logarithmic, stationary and decline phases. The time of incubation after which the greatest concentration of virus is attained varies with the infectious agent. For example, the vaccinia virus, when cultivated in the presence of chick embryonic tissues at 33° C., reaches a maximal titer on the fifth day of incubation. The influenza virus grows more rapidly, reaching a maximal concentration within 72 hours. Rickettsiae grow slowly and titers are greatest after a week (5). In roller tube cultures, titers of viruses vary somewhat during the period of incubation (4).

Temperature. Temperature exerts a profound effect on the growth of viruses and rickettsiae. Herpes simplex, vaccinia and infectious myxomatosis viruses grow best at a temperature of about 35° C. (6). Vaccinia virus may survive incubation at 45° C. for four days but herpes and myxoma viruses fail to withstand a temperature of 40° C. (6). Low temperatures favor growth of rickettsiae; *R. prowazeki* develops best at 32° C. (7).

Oxygen tension. The usual tissue culture procedures provide a rather high oxygen tension and this apparently favors viral proliferation. The effect of oxygen tension can be demonstrated in cultures of vaccinia virus incubated with and without vaseline seals. At low oxygen tensions, growth of the virus occurs slowly and titers are low (8). It may be anticipated that viruses will be demonstrated which will grow best at a reduced oxygen tension. Maximal growth of rickettsia occurs in tissue cultures in which cellular metabolism is at a low level (9).

Nutritional requirements. Viruses and rickettsiae tend to exhibit a high degree of host and tissue specificity. Vaccinia virus, adapted to the rabbit, will grow as well in chick as in rabbit embryonic tissues but grows poorly or not at all in mouse or rat embryonic tissues. The virus can be adapted to the mouse and then will grow as well in mouse as in chick embryonic tissues. The failure of a strain of vaccinia virus, adapted to the rabbit, to multiply in mouse embryonic tissues may be due either to the presence of harmful substances in the mouse tissue or to the lack of essential metabolites. Viral development occurs uninhibited in a mixture of mouse and chick embryonic tissues.

Information concerning the dependence of viruses on specific metabolites can be obtained by use of metabolite antagonists. A substance structurally related to a metabolite may be utilized by enzyme systems of the host or parasite in place of the metabolite. If the analogue is unable to serve the same functions as the metabolite, it may block or inactivate the activity of enzyme systems necessary for proliferation of the parasite. If the inhibitory action of the substance is counteracted by the corresponding metabolite, the analogue is designated a metabolite antagonist and it is assumed that the metabolite is required for development of the parasite.

On the basis of data available, it would appear that the purine, adenine, is essential for growth of vaccinia (10) and Russian spring-summer viruses (11). Adenine, guanine and xanthine seem to be required for multiplication of the psittacosis virus (12). Folic acid is essential for the psittacosis and meningopneumonitis viruses (13, 14, 15). Phenylalanine is required for development of the vaccinia virus (16) and methionine for the influenza (17) and poliomyelitis (Lansing) viruses (18).

These data suggest certain possibilities concerning the investigation of growth requirements of viruses and rickettsiae. Although the information available is limited, methods for exploration of the field have been developed and rapid progress can be anticipated. By use of the roller tube technique, observations can be made over prolonged periods. It should be emphasized, however, that biological relationships which prevail *in vitro* may differ greatly from those which exist *in vivo*. Therefore, parallel investigations utilizing both experimental animals and excised tissues are desirable in order to gain an understanding of the basic mechanisms governing viral and rickettsial proliferation.

Summary. Examples of the effect of certain physical and nutritional factors on viral and rickettsial growth *in vitro* have been presented. Tissue culture procedures provide an important means for the determination of biological properties of viruses and rickettsiae. Relationships demonstrated *in vitro*, however, do not necessarily apply in the living subject.

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