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THE EFFECT OF AEROBIOSIS ON ADENOVIRUS MULTIPLICATION

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Summary. — It was found that infectious adenovirus is not synthesized if the infected cells are incubated at concentrations sufficient to cause appreciable anaerobiosis, or if the respiratory metabolism of the infected cells is interfered with.

In the course of investigating certain aspects of the adenovirus (ADV)-host cell relationship it was found that ADV multiplication appeared to be dependent on the degree of aeration of the infected cells.

The strain of ADV type 1 and the HeLa cells employed, as well as the pertinent techniques of the plaque assay have been described in detail previously (Wassermann, 1962; 1965). Briefly, virus and host cells were both suspended in Eagle's Minimal Essential Medium (EMEM) with 5% γ-globulin-free calf serum (Hyland Laboratories) and 10 ml $NaHCO_3$ (7.5%) per liter. Adsorption of the virus to the cells was carried out at 2° C at low multiplicity of infection. As pointed out earlier (Wassermann, 1965), only at low multiplicities was there quantitative recovery of the statistically expected number of serum-resistant infective centers as plaque-forming units (PFU). After adsorption for 30—60 minutes, the cells were centrifuged, repeatedly washed to remove unadsorbed virus, suspended in 5 ml EMEM, and then incubated at 37° C in screw-capped 4-oz bottles. At intervals thereafter, samples were withdrawn and plaque assays performed using intact cells for an estimate of the total number of infective centers. Since ADV is not released spontaneously the cells were disrupted by repeated freezing and thawing and assayed for intracellular virus.

Infected cells at a density of 4×10^6/ml failed to show a demonstrable rise in intracellular virus within 24 hours, and at 36 hours there were only about 10 PFU/cell. Similar preparations, but at cell density of 1×10^6/ml, began to yield newly synthesized virus at 16 hours and reached yields of more that 2000 PFU/cell in 36 hours. These findings suggested the possibility that unfavourable conditions, such as anaerobiosis, might have prevailed in the bottles in which the cell population was very dense. In this connection Green and Piña (1963) reported lower ADV titers from dense suspensions of infected cells than from more dilute ones. It is also known that highly concentrated host cell suspensions cause a delay in bacteriophage synthesis and that simple dilution can reverse this effect (Adams and Wassermann, 1956). Gifford and Syverton (1957), in a study of anaerobic and aerobic growth of poliovirus in HeLa cells, had found that in the absence of oxygen, intracellular virus development was slowed down but not completely inhibited. The effect of oxygen was also found to be pronounced in the growth of Newcastle disease virus, several arboviruses, and vaccinia virus (Baron *et al.*, 1961), with all of these viruses requiring more or less aeration.

In order to investigate the ADV system further, a series of adsorption tubes was prepared, each with 4×10^6 cells/ml. The were infected with ADV 1 and, after removal of the unadsorbed virus, some of the tubes were closed with rubber stoppers and others with loosely fitted aluminium caps. The tubes were incubated at 37° C, either in stationary racks or on a roller drum.

Table 1. The effect of conditions of incubation on the virus yield from infected cells*

Tube closure or container	Manner of incubation at 37° C	Yield from disprupted cells (PFU)				
		2 hours	6 hours	16 hours	20 hours	24 hours
Aluminum cap	stationary	3.6×10^3	2.8×10^3	3.0×10^3	5.5×10^3	4.0×10^3
Aluminum cap	roller drum	3.3×10^3	3.1×10^3	6.9×10^3	5.8×10^5	1.1×10^6
Rubber stopper	stationary	4.1×10^3	4.3×10^3	1.1×10^3	1.0×10^3	2.2×10^3
Rubber stopper	roller drum	4.1×10^3	2.1×10^3	6.7×10^3	5.5×10^5	2.1×10^6
Petri dish	stationary (6% CO_2)	1.8×10^3	2.1×10^3	9.5×10^2	1.8×10^5	1.5×10^6

* Cell density = 4×10^6/ml.

A shallow layer of fluid containing the infected cells was incubated in a plastic Petri dish at 37° C in an atmosphere of 6% CO_2 in air. The plastic plate was of bacteriological grade in order to prevent attachment of the cells. It is clear from the data shown in Table 1 that incubation on a roller drum or in shallow layer in CO_2 and air resulted in the appearance of intracellular virus between 16 and 20 hours, whereas incubation under relatively static conditions prevented production of infectious virus. It is also evident that agitation of the infected cells was not essential, as the virus yield from the cells in the Petri plate which had been held stationary was comparable to that from the rolled tubes. Rather it appears as if the oxidation-reduction potential prevailing in the agitated tubes and in the plate was conducive to virus development. To test this, 3 tubes were equipped in such a manner that gas could be bubbled through the medium. ADV-infected cells were introduced into the tubes. One was gassed with air, the second with 6% CO_2 in air, and the third with nitrogen. The fluids were all kept adjusted to pH 7.2 throughout the incubation period. After 24 hours at 37° C, the virus content of the tubes was assayed. Normal yields of virus were obtained from the cells exposed to air or CO_2 in air, but no demonstrable increase in virus titer was shown in the presence of nitrogen. In fact, a loss of infective centers was noted in these tubes. In another experiment, designed to study the effect of shutting down the respiratory metabolism of the cells, HeLa cells were infected in the presence of 10^{-3} M sodium cyanide. Adsorption took place at 2° C for 50 minutes after which the cells were centrifuged, washed, and resuspended in EMEM. One half of the preparations were then incubated at 37° C on a roller drum in the presence of cyanide. The other half were similarly incubated but without cyanide. After 24 hours the cells were disrupted and assayed for intracellular virus. The results are shown in Table 2. Sodium

Table 2. Virus multiplication in the presence of 10^{-3} M sodium cyanide

NaCN present during		Number of infected cells (PFU/ml)	PFU yield/infected cell
Adsorption*	Incubation		
+	−	1.9×10^3	632
+	+	2.9×10^3	none demonstrable
−	+	1.9×10^3	none demonstrable
−	−	1.8×10^3	880

* The input for all preparations was 4×10^6 HeLa cells and approximately 2×10^3 PFU/ml.

cyanide had apparently no effect on the adsorption of the input virus, but maturation into infectious virus was blocked. All of these experiments point to the conclusion that aerobic respiratory metabolism of the infected host cells plays an essential role in ADV multiplication.

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