COMPARATIVE CHARACTERISTICS OF THE HISTOCHEMICAL AND BIOCHEMICAL CHANGES IN TISSUE CULTURES INFECTED BY ADENOVIRUSES

D. B. Golubev, S. Ya. Zalkind
A. A. Selivanov, I. S. Deryabina, A. M. Fokina,
and E. P. Zvereva

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Several studies have been made of the histochemical characteristics of cells of epithelial origin during the reproduction of laboratory strains of adenoviruses in them [3]. However, there is no account in the literature of the histochemical features of the action of strains of adenoviruses which have modified their biological properties after repeated passages on a culture of fibroblasts [5, 6].

In the present investigation cultures of epithelial cells and fibroblasts from chick embryos were studied after infection with ordinary and "fibroblast" strains of adenoviruses, by means of various histochemical and biochemical methods.

EXPERIMENTAL METHOD

HeLa cells infected with a laboratory strain of type 3 adenovirus (strain No. 32), cells of chick embryonic fibroblasts (CEF) infected with "fibroblast" strain (No. 3337) of type 3 adenovirus, and HeLa cells infected with strain No. 3337 were studied.

The method of cultivation of the cells, of infection with the viruses, and of estimating the virus reproduction was described in an earlier report [4]. The tissue cultures were infected with 1000 TCD_{50} of the corresponding viruses.

Layers of the control and infected tissue were grown on cover slips and investigated in the natural state in the luminescence microscope after staining with acridine orange [2]. Parallel test samples were fixed with 10% Carnoy's solution and then stained by Brachet's method [10] and for glycogen by Shabadsh's method [7].

The activity of aldolose [9] and phosphohexoisomerase (PHI) [8] in the culture fluid was determined.

EXPERIMENTAL RESULTS

In the experiments of series I histochemical and biochemical studies were made of HeLa cells infected with type 7 adenovirus adapted to this tissue. A typical adenovirus infection was revealed. A cytopathic action (CPA) was found 48 h after infection.

Luminescence microscopy with acridine orange revealed characteristic nuclear changes (Figs. 1 and 2). Estimation of the aldolase and PHI activity showed no changes in the culture fluid of the infected samples.

Staining for glycogen showed the first differences between the control and infected cells 20 h after infection. At this time 16.2% of the cells in the control samples were intensively stained, compared with 61.8% in the experimental. The differences persisted later, although they were less marked.

Differences in staining for RNA began to appear 4 h after infection. At this time the number of intensively stained cells in the control culture was 28.8% compared with 69% in the infected, whereas 20 h after infection the corresponding values were 30.3% and 64.8%.

Laboratory of Cytopathology, Moscow Research Institute of Virus Preparations; Laboratory of Virus Preparations, Leningrad Research Institute of Vaccines and Sera; and Department of Virology, Institute of Experimental Medicine, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR, G. V. Vygodchikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 5, pp. 44-48, May, 1967. Original article submitted December 15, 1965.

TABLE 1. Changes in Enzymes in the Liquid Phase of a Culture of Chick Embryonic Fibroblasts Infected with 1000 TCD_{50} of Type 7 Adenovirus Adapted to this Tissue (Strain No. 7337)

Index	Time after infection, in hours				
	48	96	144	192	240
CPA	—	—	—	+	+++
Aldolase activity (in μg enzyme)	4,0/4,0	4,3/4,2	5,0/5,2	6,4/15,2	4,2/19,2
PHI activity (in μg fructose)	8,7/9,3	12,0/10,0	12,1/19,0	13,1/27,0	14,0/24,2

Note. Numerator—activity of control sample, denominator—activity of infected sample.

TABLE 2. Results of Staining of Control HeLa Cells and of HeLa Cells Infected with Type 7 Adenovirus for Glycogen and RNA (Number of Intensively Stained Cells, in % of Total Number of Cells Examined)

Culture	Staining reaction	Time after infection (in hours)		
		144	192	240
Control	for glycogen	22,4	17,6	16,0
	for RNA	14,4	16,5	19,7
Infected	for glycogen	48,4	54,2	58,4
	for RNA	64,2	63,3	60,9

In the experiments of series II a culture of chick fibroblasts infected with type 7 adenovirus (strain No. 7337) adapted to this tissue was studied. A CPA was not detected until 192 h after infection. Outwardly it was indistinguishable from the spontaneous degeneration of the control culture.

During luminescence microscopy no changes were found in the character of the localization of nucleic acids. The CPA took the form of a disturbance of the integrity of the cell layer, although in each part of the layer the morphological and histochemical characteristics remained unchanged.

No differences between the control and the infected cultures were found likewise when the cells were stained by Feulgen's method.

Clearly defined changes in the enzymes were found, and their character is shown in Table 1.

The results given in Table 1 are typical of the reproduction of A. A. Selivanov's [4] "fibroblast" strains of adenoviruses in a CEF culture.

No differences were found in the intensity of staining of the cells for glycogen between the experimental and control cultures.

Staining for RNA revealed some differences between the control and the infected cells in the samples obtained 96 and 144 h after infection: in the control culture the number of intensively stained cells 96 h after changing the medium was 23.1%, rising to 34.8% after 144h. The corresponding values for the infected cultures were 49.3% and 43.6%.

In the experiments of series III, HeLa cells were infected with type 7 adenovirus (strain No. 7337) adapted to CEF tissue. The infecting dose of virus was 1000 TCD as shown by the results of secondary titration in chick fibroblasts.

Observations on the cultures lasted for 10 days. During this period no CPA was detected by ordinary microscopy and no histochemical changes by luminescence microscopy. The enzymic activity in the infected samples was indistinguishable from that in the controls. Clearly, therefore, no reproduction of virus was revealed by any of the methods described, whereas secondary passage of the culture fluid of the infected samples to new batches of HeLa cells led to active virus reproduction. Propagation of type 7 adenovirus adapted to CEF in HeLa cells was detected by histochemical tests for glycogen and RNA (Table 2).

As Table 2 shows, in the later periods (144-240 h after infection), the number of cells staining intensively for glycogen and RNA was much larger in the HeLa cells infected with type 7 adenovirus adapted

TABLE 3. Comparative Characteristics of Sensitivity of Various Histochemical, Miscroscopic, and Biochemical Methods of Indication of Reproduction of Adenoviruses in Tissue Cultures

Virus–cell system	CPA by ordinary microscopy	Histochemical changes by luminescence microscopy		Increase in aldolase activity		Increase in PHI activity		Staining by Brachet's method		Staining by Feulgen method		Staining for glycogen	
		1	2	1	2	1	2	1	2	1	2	1	2
Laboratory strain No. 72 of type 7 adenovirus on HeLa cells	+	+		–	–	–	–	+			+	+	
"Fibroblast" strain No. 7337 of type 7 adenovirus in CEF	±*	–	–		+	+		+		–	–	–	–
"Fibroblast" strain No. 7337 of type 7 adenovirus in HeLa cells	–	–	–	–	–	–	–	+	–	–	–	+	–

Legend: 1) results of use of the particular method of investigation if these results were discovered before the development of a CPA visible by ordinary microscopy; 2) if they were discovered at the same time as the CPA.

* In this case the CPA developed late and could be accepted as a method of indication of virus reproduction only with considerable reservations.

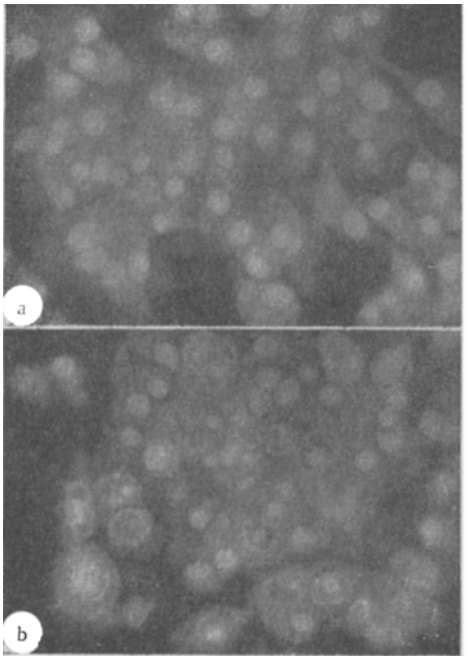

Fig. 1. HeLa cells 48 h after infection with type 3 adenovirus. a) Control tissue (48 h after changing the medium); b) infected tissue. Acridine orange. Luminescence microscopy.

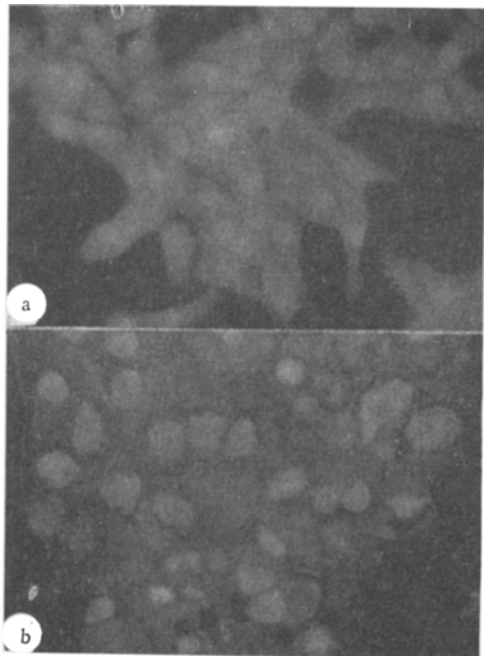

Fig. 2. HeLa cells 72 h after infection with type 3 adenovirus. a) Control tissue (72 h after changing the medium); b) infected tissue. Acridine orange.

to CEF than in the control cultures. In the earlier periods of the investigation no differences were found between the control and the experiment. The differences observed in the later histochemical reactions were the only signs of reproduction of the virus apart from the direct isolation of the virus after passage to HeLa cells.

It may be concluded from these results that each individual virus–cell model possesses its optimal methods for indicating virus reproduction. As Table 3 shows, for ordinary laboratory strains of adenoviruses all the microscopic and histochemical methods of investigation fall into this category, whereas the enzymic changes do not reflect the process of virus reproduction taking place in the cells. On the other hand, the reproduction of "fibroblast" strains in a CEF culture is best indicated by enzyme tests. Finally, during reproduction of these strains in HeLa cells, changes in the cells can be found only by histochemical methods, such as staining by Brachet's method and for glycogen.

The evaluation of the changes in cells infected with adenoviruses is thus directly dependent both on the properties of the virus–cell system and on the characteristics of the individual method used.

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