development of discrete foci of filamentous aggregates in infected tissues has never been observed in influenza viruses. Although morphogenesis of the paramyxoviruses has been assumed to occur only in the cell cytoplasm, filamentous tubules located within the nuclei have been observed in cultured cells infected with measles virus (*6*), and intranuclear inclusions have been found in tissues infected with bovine parainfluenza 3 virus (*7*). It is therefore not possible to identify the virus type of myxovirus solely by morphology.

Nevertheless, it seems reasonable at this time to predict that human chronic polymyositis is a disease of virus etiology, most likely caused by a member of the myxovirus group. It is conceivable that the existence of intranuclear and intracytoplasmic clusters of filaments in the three muscle tissues obtained during a period of 1½ years may represent a persistent chronic viral multiplication. Such a chronic viral infection has recently been suggested for herpes simplex virus infection in man (*8*). It is rather surprising to see so little evidence of degeneration of muscle fibers, the nuclei of which harbor filamentous aggregates. Signs of degeneration are seen constantly when intracytoplasmic aggregates exist. It is therefore logical to assume that morphogenesis of these filaments, which presumably are ribonucleoprotein, is initiated within the sarcolemmal nuclei and not in the cytoplasm. This may also indicate a well-balanced host-parasite relation that eventually will culminate in a total upset of the overall metabolism of the host cells. Because chronic polymyositis is commonly associated with such chronic diseases as carcinomatosis or collagen diseases, the possibility of patients' acquiring certain immunological deficits, and hence virus infection, should be considered. Although it is unlikely, it is entirely possible that these filaments, or an incomplete form of virus, represent a latent virus encountered by chance in this particular patient. If this is the case, more electron microscopic studies on muscle tissues from patients with polymyositis should be made to clarify the issue. Virological studies on the muscle tissue are now in progress.

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11 September 1967

Transforming Activity of Green Monkey SA7(C8) Adenovirus in Tissue Culture

Abstract. *Simian SA7 (C8) adenovirus can effect transformation of mouse, rat, and hamster cells in tissue culture. The transforming activity of the virus was more pronounced in culture medium containing 0.1 millimolar $CaCl_2$, a lower concentration than that usually used. The transformed hamster cells were tumorigenic in hamsters.*

The transforming activity of oncogenic adenoviruses has not been adequately studied. Only the transforming activity of human type 12 adenovirus in hamster (*1*), rat, and rabbit (*2*) kidney cells and rat embryo (*3*) cells has been described. Freeman *et al.* (*3*) have shown that the transforming activity of this adenovirus is more pronounced when the concentration of calcium ions in the maintaining medium is lowered.

The strain C8 of green monkey SA7 adenovirus (*4*) is oncogenic for hamsters (*5*). We have studied the transforming activity of this agent in cultures of embryonic skin and muscle of hamsters, C3H mice, Wistar rats, and humans, and in cultures of kidney tissue from suckling mice and hamsters.

The C8 adenovirus was supplied by Dr. H. Malherbe. The virus was grown in green monkey kidney tissue maintained in medium No. 199, to which bovine serum (2 percent) had been added. When assayed in the same tissue culture by the plaque method (*6*), the

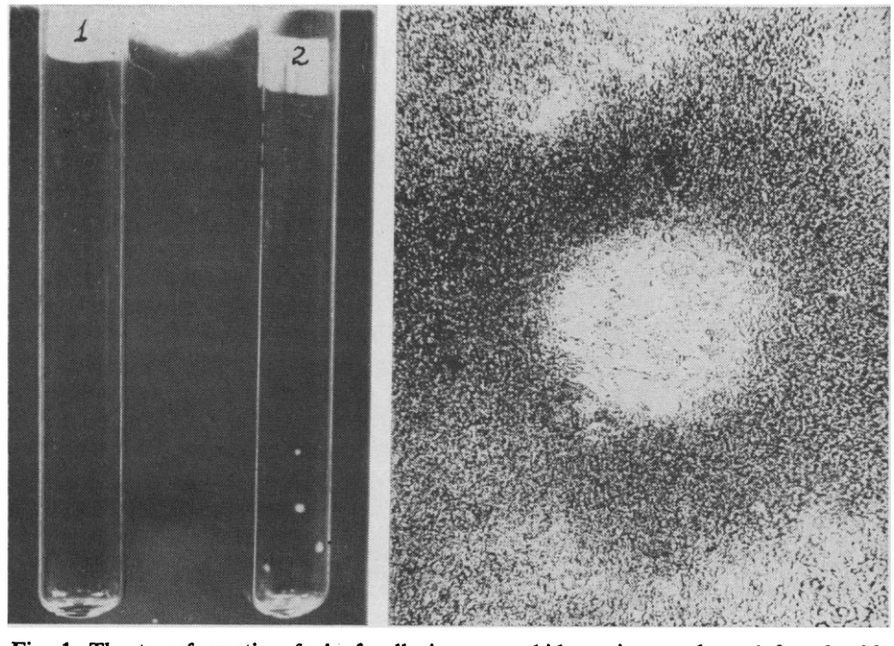

Fig. 1. The transformation foci of cells in mouse kidney tissue culture infected with SA7 (C8) adenovirus. (Left) Noninfected (1) and an infected (2) tissue culture tube (four foci are visible); (right) transformation focus with destruction of the central part. (× 105)

Table 1. Transformation of cells in tissue cultures infected with SA7 (C8) adenovirus. The maintenance medium contained 0.1 m*M* $CaCl_2$. The results are expressed as ratio of the number of tubes showing foci of transformation to the total number of infected tube tissue cultures.

Tissue culture	Transformation in culture after infection			
	Virus cultures			No virus at 9 to 11 weeks
	4 to 6 weeks	7 to 9 weeks	10 to 11 weeks	
Human embryo	0/10	0/10	0/10	0/7
Mouse kidney	2/6	3/6	4/6	0/8
Mouse embryo	0/11	7/11	10/11	0/8
Hamster embryo	0/5	2/5	4/5	0/15
Hamster embryo	7/14	12/14	14/14	0/11
Rat embryo	3/3			0/7

virus-containing material had a titer of $10^{5.5}$ plaque-forming units (PFU) per 0.2 ml. It was not contaminated by SV40 virus, as determined by tests for SV40 T-antigen, and infectivity.

Monolayer cultures of trypsinized embryo and kidney tissues were prepared in test tubes by the usual method. The growth medium consisted of Hanks's solution with the addition of lactalbumin hydrolyzate (0.5 percent) and bovine serum (5 percent) added. To date, we have not found bovine serum to contain inhibitors for the C8 virus. The monolayers were infected after confluence was reached, with 0.2 ml of undiluted virus-containing material per tube. Infected and control cultures were maintained in medium No. 199 containing bovine serum (10 percent), and with 1.3 or 0.1 m*M* $CaCl_2$. The medium was changed twice a week.

In cultured mouse kidney tissue, transitory foci of cytopathic changes appeared during the 1st week after infection, but later the monolayer regained its normal appearance. At the end of the 1st month, foci of increased cell growth appeared in several of the infected tissue cultures maintained in the medium with 0.1 m*M* calcium (Table 1). By day 40, solid, white, round spots were seen macroscopically in the tubes (Fig. 1, left). In many cases the spots were surrounded by a less-solid border. Microscopically, the foci consisted of round cells that showed a marked tendency to pile up. The rim of the foci consisted of epithelium-like cells. In a number of foci the center fell out, giving them a ring-shaped appearance (Fig. 1, right).

In cultures maintained in the medium containing 1.3 m*M* calcium, identical foci developed, but later and in smaller numbers (Table 2). In these cultures the entire focus tended to become detached from the tube.

In cultured hamster kidney tissue, the C8 adenovirus induced a marked cytopathogenic effect, and only scattered areas of healthy cells remained. Transformation of these cells was not observed.

The C8 adenovirus did not produce any distinct cytopathogenic effect in the cultured skin-muscle tissues of hamsters, rats, mice, and human embryos. In cultures of human embryo cells no morphological transformation was observed. Only traces of infectious virus were found in the culture fluid, but virus persisted for at least 5 weeks after infection.

The formation of characteristic cell foci, similar to those in cultures of mouse kidney tissue, was seen in the cell cultures from hamster, mouse, and rat embryos (Table 1). The number of typical foci of transformation varied from one to ten per tube. Only a few foci of transformation developed in cultures maintained in medium with 1.3 m*M* calcium (Table 2). Virus could not be recovered from any of these cultures after the 2nd week. Control tissue cultures usually degenerated during the 3rd month; and transformed foci were never observed.

Table 2. The influence of $CaCl_2$ concentration in the medium on the development of transformation foci induced by the SA7 (C8) adenovirus.

$CaCl_2$ in medium (mmole/liter)	Transformation foci		
	(No./total)*	Av. No. per tube	Time of appearance (weeks)
	Mouse kidney		
0.1	4/6	5.25	5
1.3	3/8	1.0	8
	Mouse embryo		
0.1	10/11	3.7	9
1.3	0/8	0	
	Hamster embryo		
0.1	4/5	2.6	7
1.3	1/4	1.0	9
	Rat embryo		
0.1	3/3	8.0	5
1.3	2/4	2.0	6

* The numerator is the number of tubes showing foci of transformation by the end of observation period (10 to 11 weeks after infection). The denominator is the total number of infected tube tissue cultures.

One line of transformed hamster embryo cells was established from cultures in the low-calcium medium. The cells were cultivated in maintenance medium containing 0.1 m*M* $CaCl_2$. At the fifth passage in vitro, 10^7 cells were inoculated subcutaneously into each of five adult hamsters. Two to three weeks later, rapidly growing tumors appeared in all animals at the inoculation site. Histologically, the tumors were undifferentiated sarcomas that were very similar to the primary subcutaneous tumors induced in hamsters by adenovirus C8 as well as other adenoviruses.

Thus, we have demonstrated that C8 simian adenovirus causes characteristic transformation of cell morphology and growth in cultured mouse, hamster, and rat tissues, and neoplastic transformation in the hamster cells. As in the experiments performed with human type 12 adenoivrus (*3*), the lowered content of calcium in the medium enhanced the development and growth of adenovirus-induced transformation foci.

Comparison of transformation foci induced by the C8 adenovirus with those induced by SV40 virus (large-plaque strain Rh_2 A426, originally obtained from Dr. Bernice Eddy) in the same types of tissue cultures revealed clear-cut differences in the transforming activity of these oncogenic agents. The foci resulting from adenovirus transformation are round with well-defined edges. They consist essentially of cells with a pronounced tendency to pile up; the transformed cells are similar in different kinds of tissue cultures; formation of these foci is inhibited by the usual concentration of $CaCl_2$. The foci resulting from SV40 transformation lack defined edges, and are considerably flatter; the cell morphology varies greatly from one kind of tissue culture to another (*7*). The cells are easily subcultivated, and their growth is not inhibited by the usual (1.3 m*M*) concentration of calcium.

The utilization of simian oncogenic adenoviruses as a model system may be useful in the study of the mechanism of virus-induced cell transformation.

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14 July 1967; revised 15 October 1967

Protein-Synthesizing Activity of the Anucleate Polar Lobe of the Mud Snail Ilyanassa obsoleta

Abstract. *Polar lobes of eggs of the mud snail* Ilyanassa obsoleta, *detached at the "trefoil" stage of the first cleavage, are capable of incorporating labeled amino acid into protein. The rate of incorporation per unit volume is about half that of the whole egg. The ability to incorporate at a similar rate persists for at least 24 hours after isolation. The sum of the incorporation of isolated lobes and lobeless fragments approximates that of the whole egg. The results extend to this material (the anucleate polar lobe) evidence for long-lived messenger RNA. They suggest also that the demonstrated morphogenetic influence of the lobe, which is exerted primarily during cleavage, may be correlated with its ability to synthesize protein.*

The eggs of annelids, mollusks, platyhelminths, and ascidians have commonly been designated as "mosaics," in that the blastomeres of the early cleavage stages have been thought to be developmentally fully specified, the regional specification applying also to the uncleaved egg (*1*).

The supposition of "mosaicism" is, however, not quite justified. For example, in the oligochaet annelid *Tubifex* (*2*), in the polychaet annelids *Chaetopterus* (*3*), *Nereis* (*3*), and *Sabellaria* (*4*), and in the mollusk *Cumingia* (*3*) double monsters can be obtained from a single egg by simple procedures that equalize the normally unequal first cleavage. Also, whole embryos can be obtained from eggs in which one or more blastomeres have been removed (*3*). Further, in ascidians (*5*) two whole eggs can be fused to give a single embryo. Even in the snail *Ilyanassa,* whose eggs had been thought to be fairly strict mosaics on the basis of early experiments (*6*), more recent analyses (*7*) of developmental effects of deletion of various blastomeres or localized regions show interactive influences rather than full regional specification. Although these interactions may result often in the appearance of early determination, as in experiments with the annelid *Nereis* (*8*), basically the situation is not different from that of "regulative" eggs such as those of sea urchins and amphibia. In these, there are interactions between parts, as revealed in the initial experiments of Lewis (*9*) and Spemann (*10*) with amphibia and of Hörstadius (*11*) with sea urchins. The influences may be predominantly unidirectional as in amphibia, in which they are termed inductions, or more reciprocal, as in the sea urchins, in which they are termed gradient-system interactions.

Although so-called "mosaic" and "regulative" eggs are basically similar in developmental properties, the former often do exhibit some special features that seem useful for the exploration of regional cytoplasmic properties in relation to gene-activation and the determination of embryonic parts. In this connection there have been a number of recent investigations (*12–14*) of protein and RNA synthesis of eggs belonging to the so-called "mosaic" group.

One special feature of eggs of certain annelids and mollusks is the presence of a temporary cytoplasmic protuberance called the polar lobe at specific times during early development. In the mud snail *Ilyanassa obsoleta,* the lobe is particularly prominent at the first cleavage, having approximately one-quarter to one-third the volume of the egg and being almost fully constricted from the two blastomeres during the progress of the first division (Fig. 1). At this time it is readily removed from the egg, and this feature has permitted the earlier experimental embryological investigations (see *6, 7*) with this species.

Experiments (*13*) on the rate of incorporation of labeled leucine into protein of lobeless eggs of this species have shown that it is somewhat lower than that of the whole egg, and especially so around the 4th day, shortly before the most active period of organ formation. Determinations (*14*) of incorporation of labeled uridine into RNA by lobeless embryos showed a decrease in rate, relative to the controls, starting at the gastrula stage (about 1 day).

From these investigations and from studies on anucleate egg fragments in other species (*15*), one might infer that the isolated lobe would be capable

Table 1. Incorporation of C^{14}-labeled amino acids into protein by whole eggs, lobeless eggs, and isolated polar lobes of *Ilyanassa obsoleta.* Incubation for 2 hours at 20°C with three amino acids (2.5 μc of each per milliliter). Experiments 1 to 3: aspartic acid (164 c/mole), arginine (9.23 c/mole), and valine (195 c/mole). Experiments 4 to 9; glutamic acid 195 (c/mole), arginine (234 c/mole), and valine (195 c/mole). The a and b refer to separate tests on eggs from the same lots. Abbreviation: cpm, counts per minute.

Experiment	Time after first division (hr)	Whole eggs		Lobeless eggs			Lobes		
		Number	cpm per egg	Number	cpm per egg	Percentage of whole egg (average)	Number	cpm per lobe	Percentage of whole egg
1*	1½	90	66.4	100	60.7	92	100	7.5	11
2a*	1¼	28	80.1	28	52.1	72	28	11.7	12
b	1¼	21	82.2	21	64.6		21	7.7	
3a	1½	17	61.4	17	65.8	98	17	8.6	15
b	1½	17	73.6	17	66.1		17	12.0	
4a	2¼	14	98.9	14	75.2	85	14	13.3	14
b	2¼	14	86.7	14	82.8				
5a	4¾	23	110.1	23	104.3	105	23	13.4	14
b	4¾	23	87.0	23	103.3		23	13.2	
6	1⅔	7	59.4	7	43.1	73	14	10.7	18
7a	1½	16	78.4	20	98.7	109	19	9.2	11
b	1½	19	94.8	20	89.9		14	10.3	
8	27	16	117.2	16	113.6	97	16	8.1	7
9a†	24	10	86.5	10	106.8	104	10	9.9	14
b	20	8	105.9	8	93.0		8	17.4	

* Eggs, lobeless eggs, and lobes pooled equally from three lots. † The parallel a and b groups were from different lots of eggs.