*ISOLATION OF INFECTIOUS DEOXYRIBONUCLEIC ACID FROM SE POLYOMA-INFECTED TISSUE CULTURES**

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Several examples of ribonucleic acids isolated from purified viruses or virus-infected animal or plant tissue have proved to be infectious.[1-8] On the other hand, findings of infectious deoxyribonucleic acids (DNA) have not been substantiated. Hayes *et al.*[9] have observed leukemias in AKR-C_3H hybrid mice injected with mixed nucleic acids prepared from the leukemic organs of AKR mice. These hybrid mice, however, show a high incidence of spontaneous leukemia.[10] Latarjet *et al.*[11] have reported the induction of multiple tumors in C_3H and AKR mice injected with nucleic acid extracted from the cells of leukemic AKR mice. Elucidation of the nature of the infectious principle in these preparations is yet to be made. (In a personal communication, Dr. R. Latarjet has informed us of his recent results. The preparations appeared to lose their ability to induce tumors in mice after treatment with DNase.) There is strong evidence[12] for the presence of at least two carcinogenic viruses in AKR leukemic mice. Whether the activity of the tissue preparations is due to the presence of infective nucleic acid remains unclear. In view of the difficulties in preparing highly-purified nucleic acids devoid of other cellular or viral constituents, the most convincing evidence for the successful isolation of an infectious nucleic acid rests on its sensitivity to a specific nuclease.

The present report describes the isolation from mouse embryo tissue cultures infected with SE polyoma virus[13] of an infectious nucleic acid resistant to the action of ribonuclease (RNase), but which is inactivated by deoxyribonuclease (DNase). The intact virus is resistant to both enzymes.

The method of culturing the virus has been described.[14] In brief, Swiss mouse embryo cells were grown in 32-ounce bottles, then retrypsinized and grown in 2-ounce prescription bottles. The maintenance fluid consisted of medium 199 and 2 per cent horse serum with the addition of 3.5 ml of 5 per cent sodium bicarbonate solution for each 100 ml of fluid. At the time the virus was inoculated, each bottle contained approximately 3×10^5 cells in a sheet which completely covered one flat surface of the bottle. On harvesting, the cells were frozen and thawed, then pooled, and this constituted the starting material for the preparation of nucleic acids.

The "crude virus suspension" was either an aliquot of pooled cultures used as such or concentrated tenfold by lyophilization. After storage from 1 to 24 hours at 4 to 6°C, aliquots of 50 ml were dialyzed against 3 liters of 0.02 *M* phosphate buffer, pH 7.2, for 24 hours. For the concentrated virus suspensions, a 48-hour period of dialysis, with two changes of buffer, was employed. The infectious titers of the pooled virus varied between 10^{-5} and 10^{-6}, whereas that of the concentrated virus was 10^{-7}.

Two methods of preparing nucleic acids from the tissue cultures were employed:

Method 1: This procedure followed in general that described by Gierer and Schramm.[1] To 50 ml of dialyzed crude virus suspension, an equal volume of 80

per cent phenol, by weight, was added, a fresh bottle being opened for each experiment. (Mallinckrodt, St. Louis, Mo., liquefied phenol, 88 per cent, analytical reagent, was adjusted with 0.02 *M* phosphate buffer, pH 7.2, to obtain the desired concentration.) The virus-phenol mixture was shaken vigorously for 15 minutes at 20°C by machine, and then centrifuged at 3900 *g* at 0°C for 5 minutes. The upper, aqueous, phase was removed and treated with 75 per cent phenol in a similar fashion two further times. The final aqueous layer was freed of phenol by ten extractions with equal volumes of peroxide-free ether at 0°C. The dissolved ether was evaporated in a stream of nitrogen at 0°C.

Method 2: This procedure was an adaptation of that described by Kirby.[15] Solid sodium-*p*-aminosalicylate (6% by weight) was added to the crude virus suspension, which was then shaken with an equal volume of 88 per cent phenol at 20°C for 15 minutes. The extraction was continued by magnetic stirring for 4 hours at 6°C. After centrifugation at 3900 *g* for 5 minutes at 0°C, the remaining procedure followed closely that described for Method 1.

This method was applied to uninfected cultures for the preparation of control nucleic acids.

When tested in tissue cultures, reproducible cytopathogenic effects were obtained in several series of experiments with concentrations varying between 3 and 30 μg of mixed nucleic acid per ml. Intact virus was later identified in these nucleic acid-inoculated cultures by demonstration of cytopathogenic changes and the ability of the supernatant fluids to hemagglutinate[16] as well as to induce tumors in injected animals.[13, 17]

The following conditions appeared to be optimal for the demonstration of nucleic acid infectivity in tissue cultures: (1) removal of the culture medium from the cell sheet followed by two washings with 0.14 *M* NaCl in 0.02 *M* phosphate, pH 7.2. and removal of the washings prior to inoculation of the nucleic acid; (2) adjustment of the nucleic acid solutions to 0.5 or 0.8 *M* NaCl in 0.04 *M* phosphate, pH 7.6; (3) addition of 2.0 ml of nucleic acids (*ca.* 3 to 30 μg/ml as estimated from absorbancy at 260 mμ) to each 2 oz. bottle containing about 3×10^5 cells; (4) incubation of the mixture at 34°C for 20 minutes; and (5) removal of the nucleic acid solutions before adding fresh culture medium. These procedures were fashioned after those used by Alexander *et al.* for the demonstration of infectivity of RNA from polio virus in HeLa cell monolayers.[3]

The results from two typical experiments are presented in the table. Comparable results were obtained with nucleic acids prepared by both procedures. The cytopathogenicity of two specimens before and after treatment with RNase and DNase may be compared with those of the crude virus from which the nucleic acid was prepared. (RNase from Sigma Chemical Co., St. Louis, Mo., 5X crystallized; protease-free. DNase from Worthington Biochemical Corp., Freehold, N.J., 2X crystallized; used in the presence of 0.005 *M* $MgSO_4$.) Uninfected cultures and nucleic acids prepared from them are shown as controls.

Exposure to RNase or DNase (3 to 30 μg/ml final concentration) did not affect the infectivity of the "crude virus," nor was the infectivity of the nucleic acid preparations affected by treatment with RNase for 15 minutes at 20°C. In contrast, exposure of the nucleic acid preparation to equivalent concentrations of DNase for an equal time completely destroyed the infectivity. The last four columns of the

INFECTIVITY OF SE POLYOMA VIRUS NUCLEIC ACID AND SE POLYOMA VIRUS FOR MOUSE EMBRYO CELL CULTURES AND FOR HAMSTERS

Method	Preparation	Days on which mouse embryo cell cultures showed cytopathogenicity[a] — 1st tissue culture passage							Days on which mouse embryo cell cultures showed cytopathogenicity[a] — 2nd tissue culture passage						Hamsters injected with 1st or 2nd tissue culture passage fluid — No. injected	No. lost	No. with tumors	Period of observation (days)
		7	14	21	28	35	42	63	7	14	21	28	35	42				
I	Nucleic acid held at 20°C for 20 min.		2						0	2					11	0	11	18
			±	3	4													
	treated with DNase		0	0	0				0	0	0	0	0	0	11	0	0	67[c]
	treated with RNase (3 μg enzyme/ml at 20°C for 15 min.)		3	3	4				0	0	3	4			12	5	7	28
II	Nucleic acid[b]	1	1	3					3	4					10	3	7	18
	treated with DNase	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	88[c]
	treated with RNase (30 μg enzyme/ml at 20°C for 15 min)	0	0	2					3	3	4				13	7	6	22
	Virus	1	4						0	2	3				7	1	6	41
	treated with DNase	1	4	4					0	3	4				13	0	12	39[c]
	treated with RNase (30 μg enzyme/ml at 20°C for 15 min.)	1	3	4					0	2	3				13	2	11	30
II	Nucleic acid from noninfected mouse embryo cell cultures	0	0	0	0				0	0	0							
	Control noninfected mouse embryo cell cultures	0	0	0	0				0	0	0							

[a] Cytopathogenicity scored: 0 = no degeneration to 4 = complete degeneration.
[b] Nucleic acid obtained from 10-fold concentrated virus infected tissue cultures.
[c] These animals remain under observation.

table contain the results of the inoculation of the fluid from the nucleic acid-infected tissue cultures into hamsters. A high percentage of these have developed tumors. The remainder are still under observation.

Detailed data on hemagglutination and results of the direct inoculation of the nucleic acid preparations into animals will be reported later together with the analytical and physical-chemical properties.

Studies on the distribution of the SE polyoma virus in mouse embryo cells by means of the fluorescent antibody technique[18] have revealed that the nuclei are first affected by the infection. Electron-microscopic examination of such cells reveals an extensive localization of the virus particles within the nuclei.[19] These findings together with the presence of a DNase-sensitive, RNase-resistant, infectious component which can be extracted and purified from a virus suspension suggest that the nucleic acid of the SE polyoma virus is DNA.

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