could substantiate their contentions with adequate statistics, all radiologists would have to be alerted to the possibility that the human body might be differentially affected by irradiation at different times of the day. Until that time, radiotherapy can be practiced with the usual safety around the clock.

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Crystallization of SE Polyoma Virus

Abtract. *Crystals have been obtained from purified preparations of SE polyoma virus. Specific infectivity measurements of the mother liquor, wash fractions, and the dissolved crystals indicate the viral nature of the crystals.*

Since the initial crystallization of poliovirus by Schaffer and Schwerdt (*1*), several animal viruses containing ribonucleic acid have been crystallized (*2*). It is the purpose of this communication to describe the crystallization of SE (Stuart and Eddy) polyoma, an animal virus that contains deoxyribonucleic acid (DNA) (*3*). This is believed to be the first report of the crystallization of a DNA animal virus. The crystallization of *Tipula iridescent*, an insect virus that contains DNA, was reported by Williams and Smith (*4*).

SE polyoma [Eddy strain 4B5 (*5*)] was grown either in tissue cultures of the mouse embryo or in cultures of kidneys of mice inoculated with the virus a few days after birth, according to the procedure of Winocour (*6*).

The cultures were harvested when most of the cells had lysed. The cells were scraped with a rubber spatula and the cells and medium aspirated into a collection flask. The plates were washed with one ml of saline-borate buffer, *p*H 9 (9 parts of 0.15*M* NaCl to 1 part of saturated sodium tetraborate), and the washings collected with the cells and medium. The alkaline cell suspension was centrifuged at 1500*g* for 20 minutes at 4°C. The virus particles contained in both the 1500*g* supernatant and the 1500*g* packed cell fractions were purified by the procedure described here (*7*).

The 1500*g* packed cell pellet was extracted with saline-borate buffer by homogenization with an all glass homogenizer. The homogenate was centrifuged in the Spinco No. 30 rotor at 10,000 rev/min for 30 minutes. The pellet was discarded. Enough 5 percent sodium deoxycholate-0.01*M* tris, *p*H 9, was added to the supernatant fraction so that a final concentration of 0.5 percent was attained and the solution was swirled at room temperature for 15 minutes. The virus in this fraction was concentrated by centrifugation in the No. 30 rotor at 30,000 rev/min for 2 to 3 hours. The pellet obtained at high speed was suspended in 0.5 percent sodium deoxycholate-0.01*M* tris, *p*H 9, by homogenization, and the differential centrifugation cycle was repeated. The pellet finally obtained at the high speed was homogenized in 5 ml of unbuffered saline and centrifuged in the Spinco SW 39 rotor at 10,000 rev/min for 30 minutes. The virus in the supernatant fraction was obtained as a clear gel-like pellet by centrifugation in the SW 39 rotor at 36,000 rev/min for 2 hours.

The virus in the 1500*g* supernatant fraction was concentrated by centrifugation in the No. 30 rotor at 30,000 rev/min for 2 to 3 hours. The pellets were pooled and suspended in 0.5 percent sodium deoxycholate-0.01*M* tris, *p*H 9, by homogenization. The virus in this fraction was then also subjected to the same differential centrifugation procedure.

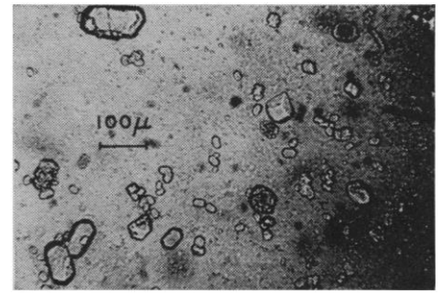

Fig. 1. Crystals of SE polyoma virus. Visible light.

Crystals were seen to form almost immediately on the addition of a few drops of water to a pellet obtained in the SW 39 rotor; this pellet being the final product resulting from purification of polyoma virus, derived initially from the 1500*g* packed cell fraction. On standing at 4°C for several days, the entire pellet had turned crystalline. The crystals appear to have the form of rhombic dodecahedra (Fig. 1).

Evidence that the crystals were com-

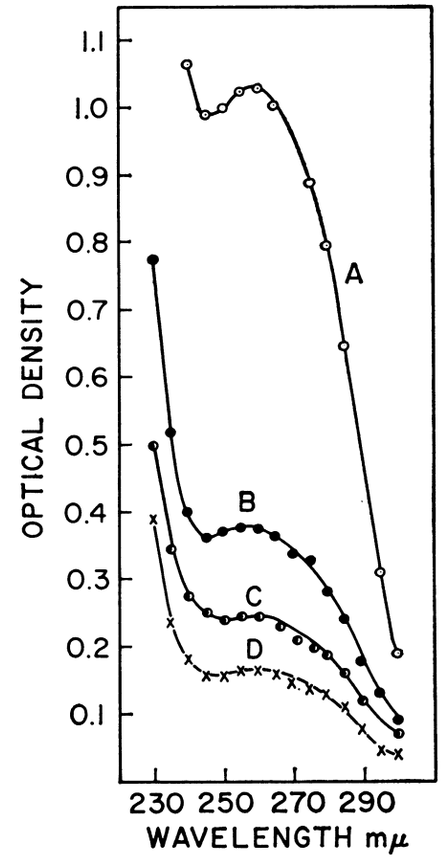

Fig. 2. Ultraviolet absorption spectra of wash fractions (Table 1) measured with the Zeiss spectrophotometer. *A*, mother liquor; *B*, wash No. 1; *C*, crystalline virus; *D*, wash No. 2.

posed of virus particles was gained by specific infectivity measurements of the solutions of washed crystals. A small aliquot of the crystal suspension was transferred to a closed capillary and centrifuged. The mother liquor was carefully withdrawn with a capillary pipet and diluted with 1 ml of 0.15*M* NaCl. The crystalline pellet, which represented approximately 1/10 of the volume of the original suspension, was washed twice with a volume of cold water equal to that of the mother liquor. Many of the crystals dissolved during the washing procedure. Each wash was diluted with 1 ml of 0.15*M* NaCl. The washed crystals were dissolved in 1 ml 0.15*M* NaCl containing 0.01*M* tris, *p*H 9. The typical nucleoprotein absorption spectra of the four fractions shown in Fig. 2 are in good agreement with the spectrum obtained by Winocour (*6*) of the "full" polyoma particle fraction purified by density-gradient centrifugation in cesium chloride. The constant specific infectivity values of the various wash solutions listed in columns 4 and 5 of Table 1 provide evidence of the comparable homogeneity and purity of these fractions.

Subsequent attempts to repeat the crystallization of polyoma virus from water have not been completely successful. Although it has not been possible to obtain the well-formed dodecahedral forms, many of the purified preparations are developing large, poorly formed, glassy crystalline masses which are closely attached to the walls of the tubes. Such masses have also formed in preparations of both "light" and "heavy" bands (*6*, *8*) of the polyoma virus obtained through further density-gradient centrifugation in cesium chloride. Studies are now underway to define more exactly the conditions required for the crystallization.

The virus has been crystallized also by the addition of saturated ammonium sulfate solutions to saline suspensions of the purified virus. The pellets obtained by centrifugation in the SW 39 rotor, originally derived from either the 1500*g* supernatant or from the 1500*g* pellet fractions, were homogenized in a small volume of 0.15*M* NaCl, and saturated ammonium sulfate solution was added until a permanent turbidity developed. The solution was allowed to stand at 4°C overnight and was then centrifuged at 1500*g*. Ammonium sulfate was added dropwise to the clear supernatant until turbidity again developed. Small crystals formed on storage at 4°C. The crystals were collected by centrifugation and the mother liquor was drawn off with a pipet. The crystalline pellet, together with about 0.1 ml of the mother liquor, was transferred to a closed capillary and the crystals washed twice with cold, 70 percent saturated ammonium sulfate to prevent solution of the crystals during the wash procedure. The washed crystals were dissolved in water. Specific infectivity values are presented in Table 2.

Table 1. Specific infectivities of wash fractions of crystalline polyoma virus (crystallized from water).

	Infectivity* (pfu/ml)	DNA† (μg/ml)	pfu/ml per OD_{260}‡	pfu/μg of DNA
Mother liquor	2.9×10^9	30	2.8×10^9	9.7×10^7
Wash No. 1	7.0×10^8	2.6	1.9×10^9	2.7×10^8
Wash No. 2	1.4×10^8	1.5	8.5×10^8	9.3×10^7
Crystalline virus	2.0×10^8	2.2	8.2×10^8	9.1×10^7

* Infectivity measured by plaque assay (*13*) and expressed as plaque-forming units (pfu). † DNA measured by quantitative complement fixation with *Lupus erythematosus* serum as antibody (*14*). The virus solutions were boiled for 10 minutes to release and denature the virus DNA prior to assay. A solution of "full" virus particles obtained by density-gradient centrifugation in cesium chloride and whose DNA content had been determined by the diphenylamine reaction (*15*) was used as a standard. ‡OD_{260} = absorbance at 260 mμ, 1 cm light path. Zeiss spectrophotometer.

Table 2. Specific infectivities of wash fractions of crystalline polyoma virus (ammonium sulfate crystallization).

	Infectivity* (pfu/ml)	OD_{260}†	pfu/ml per OD_{260}
Mother liquor	1.5×10^7	0.165	9.1×10^8
Wash No. 1	4.5×10^6	—	—
Wash No. 2	5.0×10^6	—	—
Crystalline virus	4.6×10^8	0.359	1.3×10^9

* Infectivity measured by plaque assay (*13*) and expressed as plaque-forming units (pfu). † OD_{260} = absorbance at 260 mμ, 1 cm light path. Zeiss spectrophotometer.

The specific infectivity values, based on DNA content, listed in Table 1 are approximately 10 to 25-fold lower than those calculated from data reported by Crawford *et al.* (*8*) and Winocour (*6*), respectively. These low values could, conceivably, be the result of (i) low infectivity of the starting material, (ii) inactivation of the material during purification, or (iii) presence of non-viral material in the crystals. The use of sodium deoxycholate to aid in the purification does not appear to have an inactivating effect on the virus, since essentially 100 percent of the initial plaque-forming ability can be recovered from virus preparations following two cycles of differential centrifugation under the conditions as described (*7*). Evidence for the homogeneity of the virus preparation has been provided by the essentially constant specific infectivities of the various wash fractions listed in Tables 1 and 2 and the nucleoprotein spectra which correspond closely to those published by Winocour (*6*) for the purified "full" polyoma particles. In addition, polyoma preparations purified by the procedure described contain less than 3 percent RNA relative to the DNA content [an upper limit set by the sensitivity of the orcinol procedure used (*9*)] and essentially all of the ultraviolet absorbing material has been found to be closely associated with the two hemagglutinating bands following preparative density-gradient centrifugation in cesium chloride (*7*).

Although the specific infectivities are low, their essentially constant values do indicate the viral nature of the crystals. Additional evidence for the viral composition of the crystals has been provided by Dr. Robert Langridge (*10*). Preliminary x-ray diffraction studies of a sludge of microcrystals show a "powder" pattern of about four rings which, while it cannot be unambiguously indexed, demands a unit cell of the order of 400 Å or larger. Particle diameters of 430 to 453 Å have been reported for the polyoma virus (*6*, *8*, *11*, *12*; *16*).

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Rubella Virus: Neutralizing Antibody in Commercial Gamma Globulin

Abstract. *In 19 lots of standard commercial gamma globulin from humans, the titers of antibody neutralizing rubella virus varied from 256 to 2048. The titers could not be correlated with geographic location of the donors, method of extraction, or tissue source. Samples of gamma globulin from rubella convalescents had titers of 4096. These titers were approximately 20 times higher than those in serum specimens from patients ill with natural or experimental infection. Serum antibody appears to persist for a number of years at nearly the original titers.*

Rubella virus causes a variety of congenital defects that arise during a significant proportion of pregnancies where the infection occurred during the first trimester (*1*). To prevent the development of these defects, a number of investigators have recommended the administration of human γ-globulin to exposed, pregnant women (*2*). However, Korns (*3*) and Grayston and Watten (*4*) have reported variation in the protective value of different lots of γ-globulin for individuals exposed to rubella. We now have information on the amounts of rubella-neutralizing antibody in various lots of commercial γ-globulin and relate this information to serum antibody titers in natural and experimental infection.

In the past, the assay of rubella antibody in γ-globulin and human serum was not possible. Recently, the isolation of the virus and the detection of neutralizing antibody in patients with rubella have been described (*5*). Virus grown in tissue culture has been reported to produce clinical rubella in volunteers who did not have neutralizing antibody (*6*). It was also reported that the virus could be re-isolated from these volunteers; that antibody developed following natural and experimental infection; and that clinical disease did not occur after the administration of the virus to individuals who already had antibody. The neutralization test was over 90 percent reproducible. Virus has been isolated from college students and antibody has been detected in their sera (*7*). The experimental disease is reported to have been produced in African green monkeys (*8*).

Seventeen lots of γ-globulin were obtained from manufacturers in the United States and two lots from a producer in Sweden. Fifteen of these lots were received in the lyophilized state and were reconstituted to a concentration of 16.5 percent with Hanks balanced salt solution and stored at 4°C. The other lots (D and F), containing merthiolate (1:10,000) as a preservative, were prepared for clinical use in the liquid form by the manufacturer. Of these, two were produced in this country and contained 16.5 percent γ-globulin. The remaining two lots were prepared in Sweden at a concentration of 12 percent γ-globulin. One of these latter lots was identified as Swedish convalescent rubella γ-globulin and was derived from plasma collected from military recruits approximately 4 months after clinical illness (*9*). An additional special lot of γ-globulin from rubella convalescents was prepared for reference purposes with plasma obtained from four volunteers 2 months after they developed clinical rubella as a result of the intranasal administration of 100 $TCID_{50}$ (tissue culture infective dose—50 percent effective) of safety-tested RV strain rubella virus (*10*). These volunteers were among those participating in studies similar to those reported previously (*6*). Initially, the toxicity of the merthiolate contained in the lots prepared for clinical use was considered a potential source of difficulty in the tissue-culture systems needed for the titrations. However, in practice, it became apparent that the high titers of antibody encountered in the tests with γ-globulin permitted sufficient dilution of the preservative so that this material did not interfere with the titrations. In addition to the γ-globulin specimens, 30 serums were collected from patients with natural or experimental rubella and stored at −20°C.

The RV strain of rubella after five passages in tissue culture was used for these investigations (*6*). Standard titrations were made with rubella virus diluted 1 to 3.2 (0.5 log). For box titrations, serial twofold dilutions of γ-globulin or serum were tested with 0.5 log dilutions of rubella virus in the range of 0.5 to 3.0 logs.

In performing the neutralization tests, heat inactivated, diluted γ-globulin or serum was combined with an equal volume of the appropriate dilution of rubella virus and incubated at room temperature for 1 hour. Then, 0.2 ml por-

Table 1. Rubella-neutralizing antibody titers of gamma globulin prepared by seven manufacturers (A, B, C, D, E, F, G). The values in parenthesis represent the concentration of the gamma globulin preparation used in the test. P, plasma; Pc, placenta; C, plasma from rubella convalescent patients; CE, experimental rubella convalescent patients' plasma.

Lot	Source	Population		Neutralizing antibody titer*
		Number	Location	
	(A) Ethanol extraction, 16.5 percent			
1	P	8,000	Japan	256
2	P	8,000	Japan	427
3	P	8,000	Japan	512
4	P	8,000	Japan	2048
5	P	8,000	Japan	2048
6	P	8,000	Japan	2048
	(B) Methanol extraction, 16.5 percent			
1	Pc	13,000	Urban east U.S.	256
2	Pc	13,000	Urban east U.S.	256
3	Pc	13,000	Urban east U.S.	341
4	Pc	13,000	Urban east U.S.	2048
	(C) Methanol extraction, 16.5 percent			
1	Pc	15,000	Midwest and west U.S.	256
2	Pc	38,000	Midwest and west U.S.	256
	(D) Salt fractionation, 16.5 percent			
1	P	2,800	East U.S.	512
2	P	2,800	East U.S.	1024
	(E) Ethanol extraction, 16.5 percent			
1	P	60	Los Angeles	256
2	P		Los Angeles, Oklahoma, Chicago	12
	(F) Ethanol extraction, 12 percent			
1	C		Sweden	512
2	C		Sweden	4096
	(G) DEAE Sephadex column			
†	CE	4	East U.S.	4096

*Reciprocal mean of three determinations by serial twofold dilutions of gamma globulin. Titers reproducible within twofold variation.
† Reference gamma globulin.