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Persistent Infection of a Rat Nephroma Cell Line with Kilham Rat Virus

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A rat nephroma cell line was found to be a carrier culture of the Kilham rat virus. Infectious virus was demonstrable only by passage of culture materials into other host systems.

During routine cultivation of a rat nephroma (RN) cell line (1), occasional cultures were noted to spontaneously degenerate. Fluids from the degenerate cultures were found to contain a hemagglutinating agent for guinea pig erythrocytes (Table 1). These agents were tested with rat virus (RV) immune serum by hemagglutination-inhibition (HI) methods as described by Kilham and Olivier (3). The HI test results (Table 1) indicate that the isolates were RV, since comparable titers were obtained with the isolates and a known RV preparation.

Two of the RN isolates were tested by virus neutralization tests against known RV antiserum. These tests were conducted in primary rat embryo cell cultures and employed from 100 to 500 $TCID_{50}$ of the isolates as determined by prior titration in rat embryo cells. Results of these tests (Table 1) also demonstrated that the agents isolated from degenerating RN cells were RV, because they were neutralized to the same degree as a known RV stock.

When no HA activity was demonstrable in fluids from normally growing RN cells, sonically treated suspensions of these cells were assayed for infectious virus by passage into both newborn hamsters and primary rat embryo cells. Five days after inoculating hamsters intracerebrally, the litters were sacrificed, and 20% suspensions were prepared of liver and kidney tissue pools. These samples were clarified by low-speed centrifugation and used as inocula for new litters.

Sonically treated RN cells were also inoculated in rat embryo cell cultures obtained from Microbiological Associates, Bethesda, Md. Seven days after inoculation, cells were removed from the glass, disrupted by sonic oscillation, and used as inocula for fresh rat embryo monolayers. Sonically treated rat embryo cells were used as a control and were handled in an identical manner.

The first passage of normal RN cell materials in hamsters yielded little or no HA activity and no deaths occurred (Table 2). However, upon subpassage into new litters, HA activity was noted in tissue homogenates, and many of the animals exhibited the characteristic disease produced by RV in hamsters (2).

Similar results were obtained in rat embryo cells in that no HA activity was detected in the first passage materials but was demonstrable in all four samples at the second passage level (Table 2). Two of these isolates were identified as RV by neutralization tests. The possibility existed that RV was introduced into the RN cells in our laboratory, where they have been employed as a host system for RV. A second sample of RN cells, supplied by Virginia Babcock of the Sloan-Kettering Institute, was inoculated immediately upon receipt into both host systems, and RV was isolated from these cells as well (Table 2).

The viral contaminant could have been present in the initial embryonal rat nephroma tissue or it may have been introduced during one of the implantation passages of the cells in rats where RV

TABLE 1. *Isolation and identification of RV from spontaneously degenerating RN cultures*

Culture	HA titer[a]	HI titer[b]	Neutralization titer[c]
1	256	1,024	Not done
2	512	1,024	256
3	512	512	Not done
4	1,024	1,024	128
Control (stock RV)	5,120	1,024	256

[a] Titers of sonic extracts of original degenerate cultures.
[b] HI titer of RV antiserum tested against eight HA units of each of the respective hemagglutinins.
[c] Highest dilution of RV antiserum that completely protected rat embryo cells against 100 to 500 $TCID_{50}$ of the respective isolates.

TABLE 2. *Isolation and identification of RV from normal RN cells*

Culture[a]	Hamster passage				Rat embryo passage		Neutralization titer[c]
	1		2		1	2	
	HA titer	Hamsters[b]	HA titer	Hamsters			
1	4	0/14	5,120	8/12	<2	256	Not done
2	4	0/11	20,480	9/9	<2	512	256
3	<2	0/18	5,120	6/10	<2	64	Not done
4	<2	0/14	10,240	11/14	<2	256	128
SK-2	8	0/13	10,240	8/11	<2	512	256
Rat embryo	<2	0/12	<2	0/11	<2	<2	Not done

[a] Cultures 1 to 4 were randomly selected from routinely propagated cells; SK-2 denotes second sample of RN cells received; rat embryo cells were used as controls.

[b] Numerator indicates number of hamsters dying or moribund; denominator is the number of newborn hamsters inoculated.

[c] Highest dilution of RV antiserum that completely protected rat embryo cells against 100 to 200 $TCID_{50}$ of the respective isolates.

is apparently ubiquitous (4). An interesting characteristic of this line is that, although most rats receiving implanted RN cells develop tumors, the regression rate is abnormally high compared to other transplantable tumors (1). Whether this high regression rate and the presence of RV in the cells are in any way related is not known.

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