palpated. Presume that the stillborn were eaten.

Summary. Female rats with bilateral microphthalmia are frequently not mated by microphthalmic males during their initial estrous cycles in the breeding cage. Several cycles may occur in the presence of males with no mating. Gestation in microphthalmic females is longer than in the normal colony females, and the litter size tends to be smaller. A higher proportion of stillborn young are born to microphthalmic females, and of the young born alive fewer survive to weaning age than is true of the young born to normal colony females.

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SV-40 Virus Growth and Cytopathogenicity in a Serial Rabbit Kidney Cell Line.** (31935)

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Simian vacuolating virus SV-40 is known to grow in primary and serial cell lines of African green monkey *(Cercopithecus aethiops)* origin(1) eliciting a characteristic vacuolating cytopathic effect (CPE). Although SV-40 virus has been shown to multiply in a variety of cell lines from other sources, it did not produce cytopathic effect useable for virus assay. However, upon protracted incubation with SV-40 virus, all the cell lines examined exhibited changes in cell morphology and growth patterns(2,3,4).

The observation by Black and Rowe(4) that primary rabbit kidney cells could thus be transformed led us to examine a serial rabbit kidney line, namely MA-111, for its response to SV-40 virus inoculation. SV-40 virus produced a fulminant cytopathic effect in MA-111 cells. This report describes some of our observations and indicates the usefulness of this cell line for the titration of SV-40 virus and for the production of SV-40 viral and CF antigen in cells from a non-simian source.

Materials and methods. Tissue culture. Primary African green monkey kidney cells, BSC-1(5) cells of low (34-65) and high (137) passage levels, and primary rabbit kidney cells, as well as a developmental cell line, MA-134 (African green monkey kidney), were obtained from Microbiological Associates, Inc.

The MA-111 cell line, also obtained from Microbiological Associates, was developed by Monroe M. Vincent* from newborn rabbit kidney cells. They are now in their 104th passage. Karyotype analysis kindly carried out by Dr. P. Price* showed that "MA-111 was heteroploid, with a small percentage of cells having 44 chromosomes all of which fell into the normal rabbit grouping." Similar observations were made by E. M. Earley† (personal communication). All cells were maintained at 34°C on Eagle's minimum essential medium(6) containing 3% agamma calf serum,‡ 100 units of penicillin/ml, 100 μg of streptomycin/ml, and 4 mM glutamine. Medium changes were done every 4 days.

Virus. SV-40 virus strain #776 (originally from Dr. H. M. Meyer) was obtained from the Laboratory of Infectious Diseases.§ It was propagated on African green monkey kidney

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TABLE I. Incubation Time* of Various Doses of SV-40 Virus in African Green Monkey and Rabbit Kidney Cell Systems (37°C).

Host species:	African green monkey					Rabbit		
Cell culture designation:	AGMK	BSC-1			MA-134	RK	MA-111	
Passage #:	0	34–65	137	197	12–47	0	6–24	60–87
Observations:	7†	12	8	2	8	32	9	8
MOI: 2 ‡		3				20	2– 3	2– 6
1.5	3	4		3	3	47–56§	3– 5	3
1	3							
0.5	3	3– 6		3–6	3– 6			3– 6
0							5–14	
—0.5				3–6	3– 6			3– 6
—1	4– 6	4– 6	6					
—1.5	6– 7	6		7	6–13			7
—2	6– 8	6– 8	8				5–14	
—2.5	7–10	7		8	7–10		7–11	7–11
—3	8–11	9	9–11					
—3.5	10–12	9–10		10	10–13		14	11–13
—4	11	11–18	18–20				14–18	8–12
—4.5	12–13	11–18	20–26	14	16–19		15–18	12–13
—5	20	20–25	25				14–18	14–15
—5.5	20–24		25–28	17–20			15–18	15–18

* Time from inoculation (days) until appearance of CPE.
† Number of replicate culture units employed per observation.
‡ Multiplicity of infection ($\log_{10}ID_{50}$) on the basis of an estimated $10^{5.5}$ cells per culture.
§ Cell transformation.

or BSC-1 cell cultures following inoculation of minimal virus doses, usually 10 infective doses per culture. Virus yield under those conditions is maximal(7).

Virus assay. Infectivity titer estimates were done in AGMK and BSC-1 cells employing 1 log dilution steps and 2-3 tubes per dilution. The cell cultures were maintained for 3 to 4 weeks. In BSC-1 cells, CPE appeared later than in primary AGMK, but endpoints were the same.

Complement fixation tests were carried out in the microtiter system‖ as described by Sever(8), employing an antiserum pool obtained from naturally infected monkeys.

Results. Various dilutions of SV-40 virus were inoculated into primary rabbit kidney and MA-111 cells. Even with multiplicities of greater than 100 there was no cytopathic effect apparent in primary rabbit kidney cultures. However, after about 7 weeks, some of the cultures exhibited patches of small epithelioid cells like the ones described by Black and Rowe(4).

MA-111 cells, on the other hand, developed a fulminant cytopathic effect. CPE was characterized by development of well defined foci of dark, rounded cells easily detected on the otherwise uniform cell sheet. As incubation time increased the foci spread, exhibiting typical SV-40 vacuolation, and progressed rapidly to complete destruction of the cell sheet (Fig. 1).

Incubation times (time until appearance of 2+ CPE) were similar to that observed in AGMK and BSC-1 cells (Table I); but CPE was more easily recognized in MA-111 cells which might explain the observation that, if induced by minimal doses, it was noticeable earlier than in AGMK or BSC-1 cells.

SV-40 virus yields from MA-111 cells approximated those of cells of simian origin and were inversely dependent on inoculum: *i.e.,* cultures given small inocula yielded more virus than cultures inoculated with higher doses (Fig. 2). A similar inverse dose-yield relationship had been observed earlier in AGMK and BSC-1 cell cultures(7).

CF antigen production also approached that in cells of AGMK origin. And, in rabbits this antigen produced antibody titers comparable to those obtained with conventional antigen preparations.

Discussion. This is the first report of cytolytic effects of SV-40 virus on non-

‖ Cooke Engineering Co., Alexandria, Va.

primate cells. That the described cytopathic effect was indeed produced by SV-40 virus was concluded from the appearance of typical SV-40 vacuolation, from the fact that this

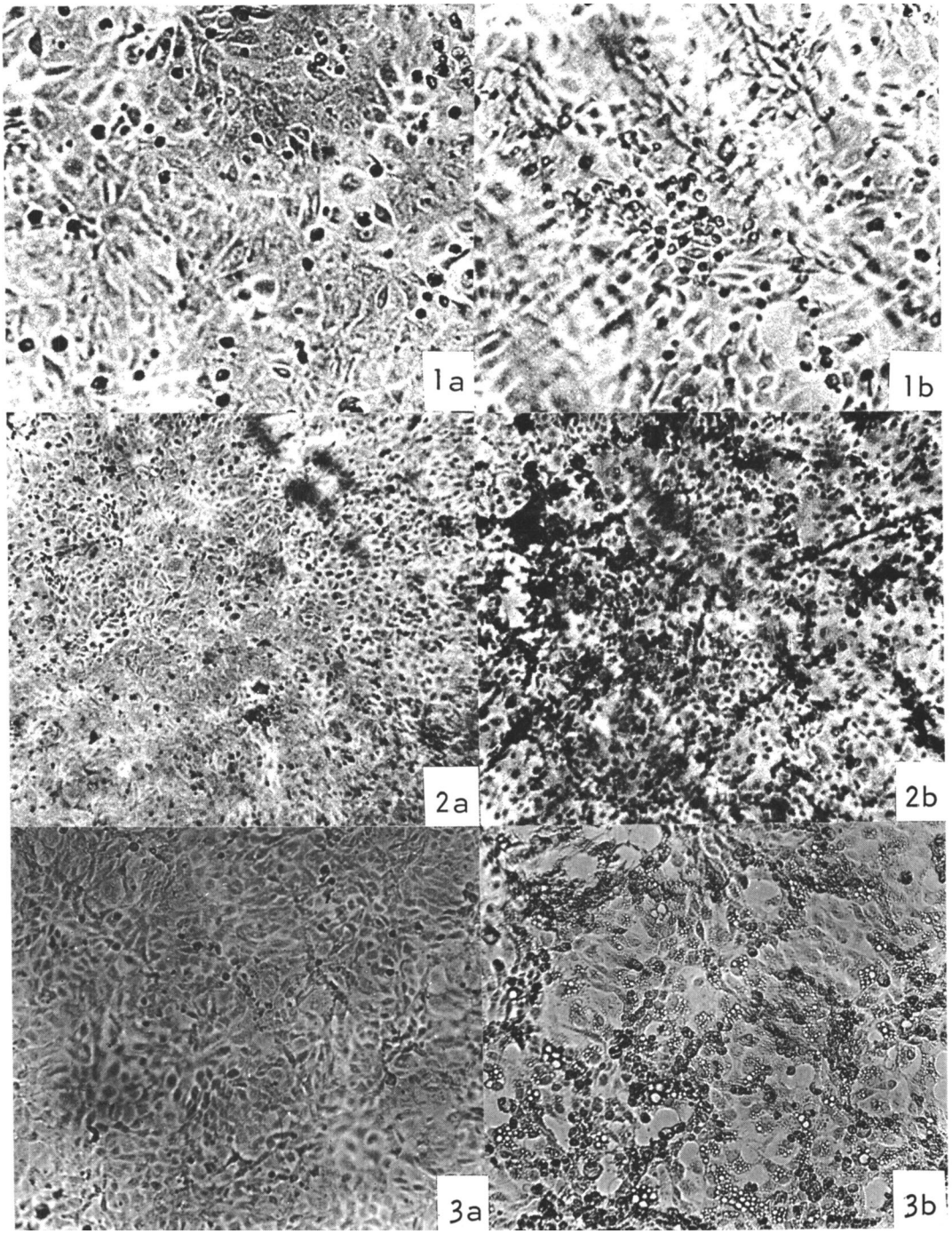

FIG. 1. Cytopathic effects of SV-40 upon MA-111 cell: 1) Top two pictures: 1a-control, 1b-early focus, 2) Middle: 2a-control, 2b-generalized granulation. 3) Bottom two pictures: 3a-control, 3b-generalized vacuolation (late CPE).

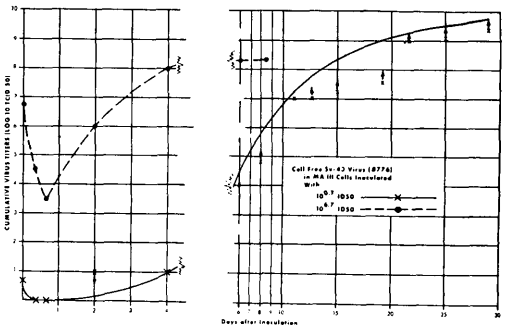

FIG. 2. Effect of dose on SV-40 virus yield.

CPE could be neutralized by SV-40 antiserum obtained from another commercial source,¶ and that viral antigen produced in MA-111 cells bound complement with SV-40 antiserum. On subpassage into AGMK cells, typical SV-40 CPE could be reproduced. Repeated limiting dilution passage in MA-111 cells did not alter the growth pattern. Antiserum produced in rabbits by hyperimmunization with MA-111 grown SV-40 antigen completely neutralized 100-1000 AGMK ID_{50} of SV-40 virus at dilutions of greater than 1/3200.

Karyotype analysis of MA-111 cells was carried out by Dr. P. Price and confirmed by E. M. Earley *(vide supra)*. Both showed chromosome numbers and chromosome configuration to be representative for rabbits. Two rabbit kidney cell lines were established in this laboratory from primary isolates. In both we could show increasing susceptibility to SV-40 virus with increasing passage levels. This enhancement of susceptibility was characterized by augmented virus yield(9), as well as by the shortening of incubation time before appearance of cytopathic effects: generalized granulation and the occasional appearance of vacuolation.

Finally, antiserum produced to MA-111 grown SV-40 antigen did not contain cytotoxic antibody to AGMK or BSC-1 cell cultures even when used at concentrations toxic for MA-111 cells. Other rabbit antiserum preparations produced against SV-40 virus grown in BSC-1 cells frequently exerted toxic effects upon cell cultures of simian origin probably due to the presence of such cytotoxic antibody.

Both karyotype analysis and this indirect evidence of host cell specificity suggest that MA-111 cells truly are of rabbit origin.

Summary. SV-40 virus elicits a fulminant cytolytic effect in a serial line of rabbit kidney cells (MA-111). One AGMK infective dose is sufficient to produce definite CPE in MA-111 within an incubation time similar to or even shorter than in AGMK cells. Virus and CF antigen yields in MA-111 approach those in cells of African green monkey origin.

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