evaluation of the genetic factor should be pursued further.

Summary. Six glucocorticoids were administered separately to pregnant rabbits of one breed over a 4-day period preceding the time of normal palate closure. Triamcinolone, dexamethasone and prednisolone regularly produced cleft palates at several dose levels below that causing litter resorption. Cortisone and betamethasone induced cleft palate sporadically and methylprednisolone did not cause cleft palate formation at any of the dose levels tested. In a second breed of rabbit higher doses of triamcinolone were required to produce cleft palates than in the first breed.

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Received May 11, 1967. P.S.E.B.M., 1967, v125.

Patterns of Antibodies to SV_{40} in Children Following the Last Booster with Inactivated Poliomyelitis Vaccines. (32336)

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The presence of infectious SV_{40} in many preparations of inactivated poliomyelitis and adenovirus vaccines(1,2), the demonstration of oncogenic properties of this virus in newborn laboratory animals(3) and its ability to multiply in, and initiate transformation of human cells *in vitro*(4) have raised the question of its possible oncogenicity in man. Two epidemiological studies designed to evaluate cancer mortality in children, in relation to SV_{40} in poliomyelitis vaccines have failed to reveal any differences in trends between vaccinated and control groups(5,6).

The availability of sequential serum samples of children following their last booster dose with formalin-inactivated poliomyelitis vaccines, afforded an opportunity to study the patterns of antibodies to SV_{40} in relation to persistence of antibodies to poliomyelitis virus. The findings of this study are the subject of this report.

Materials and methods. Serum samples. Retained sera from trials prior to the recognition of SV_{40} were available of 40 subjects inoculated with three 1.0 ml doses of formalin-inactivated poliomyelitis vaccines. The sera were collected following the last booster dose, during a period of 3-6 years before 1961.

Serum neutralization test. Sera were inactivated at 56°C for 30 minutes. Equal volumes of serum and approximately 200 $TCID_{50}$ of SV_{40}, strain 777, diluted in Eagle's basal medium, were incubated for 3 hours at 37°C, then held overnight at 4°C. Tube cultures of African green monkey kidney cells were inoculated with 0.2 ml of the serum-virus mixtures, using 4 cultures per dilution. The cells were maintained with Eagle's basal medium and 2% fetal calf serum, at 35°C, and were observed for a period of 14 days. The 50% serum neutralization endpoint was determined by the method of Reed and Muench(7). For the purpose of this study, a serum neutralizing titer of 1/10 or greater, was considered as significant.

Metabolic-inhibition test (MIT). Antibody levels to Type II poliovirus were determined by the MIT(8), modified as described previously*(9).

Complement-fixation tests (CF). Complement-fixation tests for antibodies to SV_{40} tumor antigen were performed by the microtiter technique(10). The antigen was prepared from cell packs of a virus-free hamster SV_{40}

* These tests were carried out by Mrs. Eva R. Brown, Serology Section, DBS.

TABLE I. Antibody Response in Two Vaccine Groups.

Poliovirus vaccine prepared by	Vaccine group	No. of subjects	Age range	No. serial bleedings tested	Antibody response to SV_{40}	Antibody response to Polio
Maitland culture		10	14 mo–adult	6	0*/10	10/10
Monolayer culture	A	9	5–6 yr	4	9/9	9/9
" "	B	9	" "	4	3/9	9/9
" "	C	12	" "	4	5/12	12/12

* <10

TABLE II. Geometric Mean Titers of Neutralizing Antibodies to SV_{40} and Type II Poliovirus Following Last Booster Dose with Inactivated Poliomyelitis Vaccine.

		Time post-boosters							
		1 mo		1 year		2 years		3 years	
Vaccinee No.	Vaccine	SV_{40}	Polio	SV_{40}	Polio	SV_{40}	Polio	SV_{40}	Polio
1	A	505	1280	226	640	200	113	69	80
2	"	640	>1280	80	254	40	160	40	100
3	"	120	1280	108	320	115	200	122	160
4	"	64	640	64	320	60	160	60	100
5	"	100	1280	108	160	104	100	121	80
6	"	90	640	110	320	54	160	34	100
7	"	200	1280	113	320	70	200	53	160
8	"	32	1280	32	400	28	320	28	200
9	B	40	>2560	38	320	47	320	88	320
10	C	30	NT*	30	NT*	50	NT*	40	NT*
11	C	10	160	380	160	530	160	430	100

* NT—Not tested.

tumor cell line, "EP," described previously (11).

Fluorescent antibody test. The indirect method was used. Acetone-fixed coverslip cultures of EP cells were treated with 1:10 dilutions of serum for 1 hour at 37°C, washed with saline, and covered for 1 hour at 37°C, with fluorescein isothyocyanate-labelled, goat anti-human globulin.† After staining, the coverslips were mounted and examined with a microscope equipped with appropriate ultraviolet light source and optics.

Results. We chose the antibody titers to Type II poliovirus as an indicator of successful vaccination, because this antigen elicited the most consistent immune response. The presence of neutralizing antibodies to SV_{40} and Type II poliovirus was determined in two groups of vaccinees. One group, consisting of 10 subjects, ranging from 14 months to 26 years of age, was immunized with vaccines prepared in Maitland-type cultures of rhesus kidney cells. The 30 subjects in the second group, all of kindergarten age, received vaccines prepared in monolayer cultures of rhesus kidney cells. The results of these tests are summarized in Table I. It can be noted that all vaccinees in both groups developed antibodies to poliovirus. None of the 10 vaccinees of the Maitland group developed detectable antibodies to SV_{40}, in sera obtained during a period of 6 years following their last booster dose. By contrast, approximately 50% of 30 subjects in the second group, developed a significant antibody response to SV_{40}. It is evident that the immune response to SV_{40} depended on the vaccines used, reflecting the degree of SV_{40} contaminant present.

In 11 of these 17 vaccinees, antibodies to SV_{40} were detectable for at least 3 years following their last booster dose. The geometric means of the antibody titers to SV_{40} and Type II poliovirus, are listed in Table I. Each value was derived from 2-3 separate determinations. The levels of antibody to poliovirus showed a progressive decline during the 3-year period of observation, and a similar decline of SV_{40} antibody was observed in 5 vaccinees. By contrast, vaccinees no. 3, 4, 5, 9, 10 and

† Obtained from Baltimore Biological Laboratories.

11 exhibited constant, or even slightly increased, levels of SV_{40} antibody, while the antibody to poliovirus showed the usual decline. It was of interest to determine the presence of antibodies against the SV_{40} "tumor antigens." The sera were examined at a 1:2 dilution by means of complement-fixation and fluorescent antibody tests. Sera used for fluorescent antibody tests were used unheated, and in some cases, fresh guinea pig complement was added. All these tests gave uniformly negative results.

Discussion. Our previous studies demonstrated that the majority of inactivated poliomyelitis and adenovirus vaccines, prepared in monolayer cultures of rhesus kidney cells, were contaminated with live SV_{40}, in amounts of 200-2,000 $TCID_{50}$/ml(1). Although the vaccines used in Groups A, B, and C of the present study were not available for direct testing of infectious SV_{40}, its presence can be inferred, since SV_{40} is a common and ubiquitous contaminant of rhesus kidney cells. The presence of live SV_{40} in these vaccines is strongly suggested by the pattern of the antibody response to this virus. Inactivated SV_{40} appeared to be of low immunogenicity, since 62% (13/21) of the children in vaccine groups B and C, failed to develop detectable antibodies to this virus following 3 doses of vaccine. On the other hand, vaccine A, prepared in the same manner, appeared to contain more infectious SV_{40}, since each of the 9 subjects developed relatively high antibody titers to this virus. Generally, SV_{40} antibodies showed a parallel decline, with antibodies to inactivated poliovirus. However, in 6 instances—vaccinees no. 3, 4, 5, 9, 10 and 11—SV_{40} antibody levels remained relatively constant for at least 3 years, while the corresponding antibody titers to poliovirus showed a progressive decline. This finding, although based on relatively small numbers of vaccinees, suggests the presence of a continual antigenic stimulus to maintain the constant levels of antibodies to SV_{40}. Previous reports have demonstrated that SV_{40} administered orally(12), or intranasally(13), could multiply in human beings and produce a low level antibody response. Some of the infants observed, excreted trace amounts of SV_{40} in the feces for periods up to 5 weeks. *In vitro* studies have shown(14) that the frequency of transformation by SV_{40} is directly proportional to the virus titer. A minimum of approximately 10^5 $TCID_{50}$ were necessary to demonstrate transformation of mouse fibroblasts. If the same conditions obtain in man, the chances of cellular transformation would be small. Alternatively, the possibility exists that any transformed cells produced were rejected rapidly and the amount of T antigen insufficient to elicit an antibody response. The absence of antibody response to SV_{40} following administration of poliomyelitis vaccine prepared in Maitland cultures of rhesus kidney tissue, was not unexpected, since our earlier studies indicated freedom from SV_{40} contamination in such vaccines. We have since observed that SV_{40} reaches relatively low titers in Maitland cultures of rhesus kidney tissue, and is usually completely inactivated during formaldehyde treatment‡. The observations recorded here, emphasize the need for additional serological and epidemiological surveys of selected groups of vaccinees.

Summary. The antibody patterns to SV_{40} were studied in 190 sequential sera obtained from 40 vaccinees, following their last booster dose with formalin-inactivated poliomyelitis vaccines. None of 10 subjects, immunized with vaccines prepared in cultures of minced rhesus kidney cells, developed detectable neutralizing antibodies to SV_{40}. Seventeen of 30 subjects who received vaccines prepared in monolayer cultures of rhesus kidney cells, developed significant levels of neutralizing antibodies to SV_{40}. In 6 of these cases, the antibody levels to SV_{40} remained constant for at least 3 years, while the antibody titers to Type II poliovirus showed a progressive decline. None of the sera tested contained detectable antibodies to SV_{40} tumor antigens.

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Received May 11, 1967. P.S.E.B.M., 1967, v125.

Reovirus Type 2-Infection, Cycloheximide, and Cell Death.* (32337)

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Mammalian cells infected with reovirus have been shown to produce two kinds of ribonucleic acids, an early single-stranded species, followed by a double-stranded structure which is also found in the mature virion (1,2,3). The present communication describes the effect of cycloheximide, an antagonist of protein synthesis, on the production of viral RNA and of complete reovirus particles. In addition we wish to describe the unique development of extensive cytopathology occurring only in infected cell cultures which have been treated with the antibiotic. To our knowledge, this combined action between virion and antibiotic has never been previously described.

During the course of this investigation Watanabe, Kudo and Graham(4) and Shatkin and Rada(2) reported that inhibition of the production of the two kinds of ribonucleic acids by cycloheximide was dependent on when the antagonist was added. Whereas the early addition of cycloheximide to L cells infected with reovirus type 3 was found to inhibit the production of both kinds of nucleic acids, the antibiotic when added later during the infectious cycle selectively inhibited the synthesis of the double-stranded form of viral RNA.

Materials and methods. Cell cultures. The routine growth and preparation of HeLa cells have been described previously(5). The growth and basic experimental media consisted of Eagle's basal medium (EBM) plus 10% calf serum, and EBM plus 0.2% fetal calf serum, respectively.

Virus. Reovirus type 2 (strain D-5) was grown in HeLa cell cultures and titer was determined by the immunofluorescent assay method(6). The titer in RA cells was 2.1×10^8 infectious units (IU) per ml.

Infection procedure. Monolayers of HeLa cultures were infected with an exposure multiplicity of 10 to 20 IU per cell. After adsorption for 2 hours at 37°C, unadsorbed virus was removed by washing with balanced salt solution; then the experimental medium was introduced, and the culture reincubated at 37°C.

Protein and RNA analyses. Protein synthesis was determined by exposing experimental cell cultures at various intervals to tritiated leucine (New England Nuclear Corp., specific activity 5.0 C/mM) for 15 minutes. The amount of trichloroacetic acid-insoluble radioactivity incorporated was measured in a Packard liquid scintillation spectrophotometer and expressed as counts per unit of absorbancy at 280 mμ. The synthesis of RNA was determined as previously described(7).

Antibiotic. Cycloheximide (Actidione) was generously supplied by the Upjohn Company, Kalamazoo, Michigan.

* This investigation was supported by research grant AI04685 from Nat. Inst. Allergy & Infect. Dis.

† Pre-doctoral fellow of National Science Foundation.