large fraction of the radioactivity lost in one phase of the cycle is reaccumulated in the next. This suggests that the rate of degradation of thyroid hormone in the cat is considerably higher than that which has been reported in other species. It also indicates that thyroid-renal sharing of iodide in the cat differs from that described for the rabbit by Brown-Grant *et al.* They found that approximately 90% of the iodide from degraded hormone was excreted by the kidneys in 24 hours. Clearly such a 9:1 ratio favoring renal excretion is impossible in the cat.

The longer cycle observed in the monkey may not be characteristic of this species. In fact, several complicating factors were involved. Neither temperature nor lighting was controlled, the animals were under some restraint throughout the experiments and at the beginning of each experiment they were introduced into a strange environment. It is of some interest, however, that a 2-day feeding cycle has been reported in another simian species(8).

Finally, if the fact that the 2 decerebrate cats did not show cycles proves to be the general case then it will be fair to conclude that the central nervous system participates in the timing if not the initiation of the rhythm. The site and nature of the central mechanism will be an interesting problem for future investigation. On the basis of present data the spinal cord below the 7th cervical segment may be excluded.

Summary. Cycles were observed in thyroid content of radioactive iodide in cats and monkeys. The period of the cycles in cats was approximately 24 hours. In the 2 monkeys it was longer. The cycles occurred in both species after transplantation of the thyroid and persisted in the cat following division of the spinal cord in the lower cervical region. In 3 experiments on 2 decerebrate cats no cycles were found.

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Occurrence of Adenovirus-SV40 Hybrids Among Monkey Kidney Cell Adapted Strains of Adenovirus.* (31093)

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In order to obtain adenovirus strains suitable for production of respiratory vaccines, field strains of adenovirus types 1 through 7 were adapted to rhesus monkey kidney (RhMK) tissue culture(1). It was recently found that the adenovirus 7 vaccine strain LLE46, which had become contaminated with SV40 virus during RhMK adaptation and been freed of the contamination by passage with SV40 antiserum, had formed an intergeneric "hybrid" with a portion of the SV40 genome (2-4). A proportion of the adenovirus capsids in this strain contained the SV40 genetic material which induced SV40 T antigen. The ability of the E46 virus to propagate in monkey cells was dependent on these SV40 DNA

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TABLE I. Passage Histories of Monkey Kidney Adapted Strains of Adenoviruses.

Type	Strain	Passages prior to use of SV40 antiserum (RhMK)	Passages with SV40 antiserum (AGMK)	Subsequent passages without SV40 antiserum (AGMK)	Adenovirus titer (HEK), $TCID_{50}$/ml log_{10}	Titer of SV40 virus* (AGMK), $TCID_{50}$/ml log_{10}
1	Ind. 1	8	4	1	7.0	none detected
2	Ind. 2	7	4	1	9.2	3.8
3	JF	19	10	17	>7.0	none detected
4	RN	28	10†	0	6.7	4.5
5	Ad. 75	8	4	1	8.4	2.8

* Titer of material heated at 56°C for 10 min.
† Eight of 10 passages with SV40 antiserum were made in HEK.

carriers, since nonhybrid clones from E46 did not replicate efficiently in monkey cells(5), and since SV40 infection is known to potentiate adenovirus replication in these cells(6).

It was of interest to determine if the monkey kidney adaptation of the other adenovirus vaccine strains was similarly dependent on hybridization with SV40 genetic material; types 1 through 5 were studied. All of these strains, like E46, had become contaminated with SV40 during passage in RhMK, and had been passed in African green monkey kidney (AGMK) cultures with SV40 antiserum in an attempt to eliminate the contaminant.

To establish that the adenovirus vaccine strains had become hybridized with SV40 genetic material, it was felt that the following criteria would suffice to rule out alternative possibilities, even though some of the preparations contained a small amount of SV40. The adenovirus should induce SV40 T antigen in a high percentage of human embryonic kidney (HEK) tissue culture cells within 24 hours, while an artificial mixture of SV40 and the homologous nonhybrid prototype adenovirus strain would not. The induction of SV40 T antigen should not be inhibited by SV40 antiserum, but would be inhibited by homologous adenovirus antiserum, even in the presence of heterologous adenovirus to act as a helper virus.

Materials and methods. Viruses. The adenovirus type 1 to 5 vaccine strains were those described previously(1); Table I summarizes the passage histories of the materials used in these experiments. The antiserum passages of types 3 and 4 had been made by commercial drug firms; all passages of types 1, 2, and 5 were made in our laboratory. The latter strains were passed with SV40 rabbit antiserum by the following procedure. Undiluted virus was mixed with an equal volume of a 1-5 dilution of high titer SV40 rabbit antiserum. The virus-serum mixtures were held at room temperature for 4 hours, and 0.2 ml was inoculated into tube cultures of AGMK cells. Maximal adenovirus CPE developed within 4-6 days. After 4 such passages with SV40 antiserum each strain was passed into 32-oz bottle cultures of AGMK cells, without antiserum. Virus stocks were prepared by holding the cultures until maximal cytopathogenicity developed, freezing and thawing twice, clarifying by low speed centrifugation, and storing at —70°C. Each of the 5 virus stocks was tested for infectious SV40 by heating 1 ml samples in sealed glass ampoules at 56°C for 10 minutes (to inactivate adenovirus) and titering in AGMK cultures.

Tissue culture. Primary HEK or AGMK cultures were obtained from either Microbiological Associates Inc., Bethesda, or Flow Laboratories, Rockville, Md. All cultures were maintained on Eagle's basal media with 2% agammaglobulinic calf serum, glutamine and penicillin and streptomycin.

Fluorescent antibody (FA) and complement fixation (CF) assays. Preparations for FA testing were made by mixing aliquots of adenovirus, either undiluted or diluted 10^{-1}, with equal volumes of 1:20 normal rabbit serum, homologous rabbit adenovirus antiserum, or SV40 rabbit antiserum. The virus-serum mixtures were held at room temperature for 30 minutes, and 0.2 ml inoculated into a Petri dish culture containing HEK cells growing on coverslips(7). The cultures were held at 37°C in 5% CO_2 for 20 to 24 hours, washed twice with either phosphate buffered saline pH 7.4 or Eagle's basal me-

TABLE II. Neutralization of Hybrid-Induced SV40 T Antigen in HEK by Adenovirus Antiserum.

Method of testing	Adenovirus type:	Vaccine strain + normal rabbit serum	Vaccine strain + homologous antiserum: Virus-serum mixture	Vaccine strain + homologous antiserum: Virus-serum mixture with E46⁻	Vaccine strain + SV40 antiserum	Homologous adenovirus prototype strain + SV40 virus
FA*	1	84%	4.0%	2%	85%	0
	2	47%	0.2%	0 †	25%	0
	3	90%	0	0	90%	0
	4	20%	0	0	20%	0
	5	38%	1%	0.2%	33%	0
CF‡	1	4+	0	0	4+	0
	2	4+	0	0	4+	0
	3	4+	0	0	4+	0
	4	4+	0	0	4+	0
	5	4+	0	0	4+	0

* Percentage of cells showing specific nuclear staining with SV40 tumored hamster serum.
† 0 = <0.01%.
‡ Degree of complement fixation at a 1-4 dilution of antigen tested against 4 units of SV40 tumored hamster serum. 4+ = complete fixation.

dium, and fixed in cold acetone for 10 minutes. Coverslips were stained by the indirect FA technique described elsewhere(8), using SV40 tumored hamster serum. This serum was obtained from hamsters carrying tumors induced by tissue culture cells of the THK-1 line of SV40 transformed hamster cells(9).

Antigens for CF testing were prepared by inoculating 4-6 HEK tube cultures with varying doses (25-600 $TCID_{50}$ per cell) of the virus-serum mixtures. Cultures were harvested at 48 hours by scraping cells into fluid and pooling the contents of replicate tubes. The cells were sedimented by low speed centrifugation and concentrated 10- or 20-fold by removing the appropriate volume of supernate. The resulting cell packs were frozen and thawed twice and tested in the CF test by previously described techniques(10) against SV40 tumored hamster serum. The hamster serum used in CF testing was obtained from hamsters bearing transplanted SV40 tumors. The original tumor in this line was induced by SV40 transformed hamster embryo cells(11).

To complete the criteria outlined above, the following virus-virus or virus-serum mixtures were also inoculated into coverslip and tube cultures of HEK and tested by FA and CF: 1) homologous nonhybrid adenovirus (10^5-10^7 $TCID_{50}$ of the prototype strain) with 10^7 $TCID_{50}$ of SV40 strain 777, and 2) an aliquot of the adenovirus-homologous antiserum mixture plus 10^7 $TCID_{50}$ of adenovirus 7 strain E46⁻ (a subline of E46 free of SV40 genome)(5).

Results. The results of the FA and CF testing are summarized in Table II. All 5 viruses induced FA-stainable SV40 T antigen in a high proportion of cells, and the induction of SV40 T antigen was inhibited by antiserum to the homologous adenovirus prototype but not by SV40 antiserum. Addition of heterotypic adenovirus as a possible helper did not alter the pattern of neutralization. Also, the mixtures of the prototype adenovirus strains with SV40 virus did not induce SV40 T antigen in the 24-hour test. Comparable results were obtained in the CF assays.

The pattern of intranuclear SV40 T antigen fluorescence induced by types 1, 2, and 5 differed from that described previously for E46⁺. A majority of the stained nuclei contained numerous, irregular, multifaceted inclusion bodies with very intense staining outlining each inclusion. This pattern presumably resulted from the formation of the characteristic adenovirus inclusion bodies, the inoculations having been made with doses of virus sufficient to produce large numbers of mixed infections. That this fluorescence was specific for SV40 T antigen and was not due to non-specific trapping of serum was demon-

strated by the failure of normal hamster serum to produce similar fluorescence. In contrast, types 3 and 4 exhibited the intense granular or homogeneous staining of the entire nucleus, with nucleolar sparing, as described for SV40 and E46+.

Progeny from each of these adenovirus vaccine strains obtained from either HEK plaques or limiting dilutions in HEK titrations did not induce FA-stainable SV40 T antigen in HEK. The sublines obtained from either the HEK plaques or limiting dilutions of types 1, 2, 3, and 4 failed to produce adenovirus CPE in AGMK cultures beyond the second or third passage. The subline of adenovirus type 5, however, produced complete adenovirus CPE in AGMK cells through 5 passages.

Discussion. The data presented here, plus the more definitive studies of the Ad. 7 strain LLE46, indicate that all of the 6 adenovirus vaccine strains tested are hybridized with SV40 genetic material, in the sense that these strains carry SV40 genetic material in adenovirus capsids. The SV40 contaminant acquired during passage in RhMK tissue cultures (RhMK cultures are frequently infected with this virus), was probably responsible for the adaptation of these viruses to monkey cell cultures, since the nonhybrid adenovirus recovered from these strains showed no greater ability to propagate in monkey cells than the homologous prototype. This hypothesis is somewhat incompatible with the pattern of progressive adaptation originally observed(1); if SV40 contamination were the only factor in the adaptation, a sudden increase in ability to propagate should have been seen. It is possible that some monkey-adapted mutants were selected initially, but were outgrown once their selective advantage was lost. Rubin has shown that it is possible to adapt an Ad. 4 strain to AGMK cells without participation of SV40 (B. A. Rubin, personal communication).

Although much evidence indicates that E46 is carrying only a limited portion of the SV40 genome, it is highly probable that some of the other vaccine strains carry the entire SV40 genome within adenovirus capsids. The Ad. 4 vaccine strain RN could not be freed of SV40 by intensive antiserum treatment or cloning in MK cells(12), and the bulk of the SV40 infectivity in this strain was shown to be heat inactivated at the same rate as the adenovirus(13). Similarly, the Ad. 2 vaccine strain, Ind. 2, has recently been shown to carry the infective SV40 genome in Ad. 2 capsids(14). The inability of serial passage with SV40 antiserum to rid the type 5 vaccine strain of infectious SV40 (Table I), suggests that this type also may be carrying the infectious SV40 genome.

The oncogenicity of the vaccine strains is under study; like E46(15), the Ad. 2 and Ad. 3 strains have been found to induce SV40 type transformation of hamster kidney cells *in vitro* (Rowe, W. P., and Pugh, W. E., unpublished data).

Summary. The strains of adenovirus types 1 through 5 adapted to rhesus monkey kidney cultures for use in vaccines became contaminated with SV40 virus and formed hybrids, carrying SV40 genetic material in adenovirus capsids in a manner similar to that seen in Ad. 7 strain LLE46. The ability of the strains studied here to propagate in monkey cells (with the possible exception of type 5) is dependent on the SV40 genetic material and is not due to emergence of monkey-adapted adenovirus mutants.

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Effect of Dietary Fats on Infection by *Escherichia coli* in Chicks.*† (31094)

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Ross and Adamson(1) reported that chicks deficient in essential fatty acids (EFA) developed aspergillosis more readily than those fed a diet containing corn oil. Hopkins *et al* (2) reported that chicks fed an EFA-deficient diet developed a respiratory disease while those which received the diet supplemented with soybean oil did not. The causative agent of the disease was not identified. Nagai *et al* (3) showed that EFA-deficient mice were susceptible to bacterial infections but added linoleate conferred protection. Dubos(4) reported peanut oil increased survival of tuberculosis-infected mice, while Hedgecock(5) found olive oil to be ineffective against that agent compared with coconut oil. The experiments reported here were conducted to determine if the EFA-deficient chick is more susceptible to *E. coli* infection than chicks given diets supplemented with various oils of widely varying fatty acid content and to determine the comparative activity of purified fatty acids.

Experimental. White Plymouth Rock chicks from flocks of the Poultry Disease Research Center were selected at random and placed in wire-floored electrically heated batteries. All chicks were fed the prescribed ration from the time of hatching. The basal ration was a casein-gelatin-cerelose diet described by Edwards(6). Supplementary oils: coconut, corn, menhaden and linseed, were added at the rate of 2%. The fatty acid composition of these oils as determined by gas-liquid chromatography is shown in Table I(7). Methyl oleate (18:1) or methyl linoleate (18:2) were added at the 1% level, approximately the amount present in the corn oil diet. The methyl oleate was prepared from olive oil by low temperature crystallization(8) and the methyl linoleate by the urea adduct method (9). The methyl oleate was 98% pure, containing traces of myristic acid, palmitic acid and palmitoleic acid. The methyl linoleate was 96.5% pure, the contaminant being almost entirely methyl oleate. A commercial starter ration free of medicaments was also used in one trial.

TABLE I. Fatty Acid Composition of Oils.

Fatty acid	Corn oil	Coconut oil	Linseed oil	Menhaden oil
	% Dry wt of total fatty acids			
8:0		.4		
10:0		6.9		
12:0		28.8		
14:0		24.4	.7	11.8
14:1				.4
16:0	13.5	16.3	7.7	23.4
16:1				15.7
18:0	2.9	5.1	4.7	4.8
18:1	30.8	13.8	22.0	15.8
18:2	51.1	4.2	18.2	1.5
18:3	1.7		46.7	2.4
18:4				1.6
20:3				.7
20:4				.8
20:5				9.9
22:5				1.3
22:6				9.7

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