consequence of cell division. If cell division were to be the critical event in causing either the diminution of tryptophane pyrrolase induction or the enhancement of the tyrosine transaminase induction, one would expect these events to follow the time course of mitotic activity. This does not seem to be the case: the changes in enzyme activity occur at identical time intervals after hepatectomy whether the mitotic activity is delayed (hypophysectomized rats) or normal (growth hormone treated rats).

The relative unimportance of cell division as a factor modifying the endogenous levels and the induction by cortisol of both enzymes is also emphasized by the fact that in a slowly growing tumor (5123) the tryptophane pyrrolase levels are extremely low and show no response to cortisol while in 24-hour-regenerating liver, a tissue with a much more rapid cell proliferation, the baseline level of the same enzyme is only slightly lower than in normal liver and its induction—though impaired—is still clearly present.

At present it cannot be decided whether the alterations in enzyme activities observed in the hepatoma are in any way related to the enzymic changes found in regenerating liver. The results presented here, however, allow the conclusion that neither the deletion of control mechanisms observed in hepatoma 5123 by Pitot and Morris nor the altered response of tryptophane pyrrolase and tyrosine transaminase to cortisol in regenerating rat liver is caused by cell division.

Summary. The induction of tryptophane pyrrolase and of tyrosine-α-ketoglutarate-transaminase with cortisol is altered in regenerating liver of hypophysectomized rats as compared to normal liver. While there is a decrease in induction of tryptophane pyrrolase 24 hours after hepatectomy a marked increase in the response of tyrosine transaminase activities of cortisol is found during the entire period of regeneration. The onset of mitotic activity during liver regeneration is delayed in hypophysectomized animals. By application of growth hormone it can be brought back to normal. This variation of the mitotic activity, however, has no influence on the time course of the changes in the induction of the two enzymes.

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Prevention of SV_{40} Virus Tumorigenesis in Newborn Hamsters by Maternal Immunization.*† (32537)

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Immunologic control of virus-induced cancer presents 3 possibilities, *viz.*, immunization against initial viral infection, immunization against homologous tumor-specific antigen prior to tumor appearance, or immunization

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† A. Itkin performed the statistical computations.

against metastases employing autochthonous tumor vaccine. Prevention of viral infection seems to offer the greatest promise since it removes the necessary and indispensible component for tumorigenesis irrespective of any secondary factors in induction and host immune responses which might be involved in development of clinical neoplasia. Viral tumorigenesis in experimental animal models is usually dependent upon infection at a very young age. Attempted immunization early post-partum appears impractical since the newborn is often incapable of satisfactory immunologic response and since infection of the host with the oncogenic virus might be achieved prior to development of immunity. A more satisfactory approach would seem to lie in passive immunization to protect the newborn beyond the age of high risk to cancer. Passive immunization might be achieved by postnatal administration of specific immune globulin or by appropriate immunization of the mother before conception or delivery. The latter approach appeared the more practical and was investigated in the oncogenic SV_{40} virus-hamster model system. The findings are reported here.

Materials and methods. Hamsters. Golden Syrian hamsters which were random bred in a closed colony were obtained from Lakeview Hamstery, Newfield, N. J. *Virus.* Strain VA45-54 of SV_{40} virus was passed 5 times in cell cultures of grivet monkey kidney (GMK) and titered $10^{-7.6}$ $TCID_{50}$/0.2 ml in GMK. The noninfected control fluid had the same passage history as the virus. *Immunization.* Mature female hamsters were given four 1 ml doses of SV_{40} virus or control fluid at weekly intervals by the peritoneal route. The first dose of virus or control was given in Freund's incomplete mineral oil adjuvant. *Isolation of SV_{40} virus.* The newborn hamsters were minced and homogenized in mortars with alundum to give 20% suspensions (w/v) in Hanks' balanced salt solution (HBSS). The supernates obtained following low-speed centrifugation were diluted 1:2 and 1:10 and inoculated into primary GMK cultures maintained with Eagle's basal medium (BME) containing 2% agamma calf serum. The cultures were observed for specific cytopathic effect until they began to degenerate at which time they were frozen, thawed, and passed to fresh GMK cultures with continued observation for a total of 69 days.

Serum neutralization test. The hamster sera were inactivated at 56°C for 30 minutes and were titrated for neutralizing antibody in tests employing 100 to 320 $TCID_{50}$ of SV_{40} virus per test dose. The virus-serum mixtures were incubated at 37°C for 1 hour and overnight at 4°C prior to inoculation into groups of 4 GMK cultures. The neutralizing antibody titer was calculated according to Reed and Muench(1) and was 0.1 ml of the highest initial dilution of serum which prevented cytopathology in 50% of the cultures.

Complement-fixation (CF) test. The micro-CF test(2) was employed using heat-inactivated test sera, 8 units of SV_{40} hamster tumor antigen(3) or 10% normal hamster muscle extract and 2 exact units of guinea pig complement. The titer was the reciprocal of the greatest initial dilution of serum which gave 3 to 4 plus fixation (no hemolysis was scored as 4 plus).

Experimental design. The adult female hamsters were bred approximately 1 week following the last dose of live virus vaccine and samples of blood were taken by orbital puncture 4 days after bearing the young. About ⅔ of the animals in each litter were challenged subcutaneously within 24 hours of birth in the interscapular region with 0.2 ml of SV_{40} virus diluted to contain $10^{5.6}$ $TCID_{50}$. A few unchallenged animals were decapitated 4 days after birth, the blood collected for serologic tests, and the carcass saved for attempted virus isolation. Other unchallenged animals were bled by orbital puncture 1 month later. The individual female hamsters were housed in isolation filter cages until they were discarded. The litters were also held in filter cages until they were 2 months of age at which time they were transferred to open cages. Littermates were caged together regardless of virus inoculation history since transmission of virus does not occur on contact(4). All animals were examined weekly for tumors, and all tumors were confirmed by gross or histologic examination.

TABLE I. Prevention of SV_{40} Virus Tumorigenesis in Newborn Hamsters by Maternal Immunization with Homologous Virus.

Maternal immunization	No. of litters	Tumor incidence, by days, following challenge with SV_{40} virus								Adjusted, %†
		30	140	168	182	211	225	277	364	
4 weekly i.p. doses of live SV_{40} virus	3	0/20*	0/20	0/20	0/18	0/18	0/15	0/12	0/7	0%
4 weekly i.p. doses of control fluid	5	0/27	1/22	2/21	4/21	5/21	6/20	7/14	8/14	34%

* Cumulative number with tumor/survivors (includes tumor animals).
† Percentage of animals with tumors adjusted for nonspecific deaths by standard life table procedures.

Results. Prevention of tumors by maternal immunization. The animals were observed for development of tumors for 1 year. Table I shows that 34% of the hamsters which received control fluid developed tumor while, by contrast, all of the animals whose mothers were immunized with the live SV_{40} virus were protected. *Serologic response to maternal immunization.* Table II shows that the 2 mothers whose sera were tested 4 days after giving birth displayed high neutralizing antibody titers, *viz.*, >725 and 20,480, against SV_{40} virus. The SV_{40} neutralizing antibody was present in the offspring at 4 days but was lost, in the majority of animals, by 1 month. The control animals were devoid of SV_{40} neutralizing antibody and none of the animals tested developed CF antibody against SV_{40} hamster tumor antigen or against the control antigen preparation. *Absence of* in utero *infection with SV_{40} virus.* As shown in Table III, offspring which were not challenged with SV_{40} virus and which were sacrificed 4 days after birth did not show presence of SV_{40} virus

TABLE II. Tests for SV_{40} Antibody in Mother Hamsters and Their Offspring Following Maternal Immunization with SV_{40} Virus.

Maternal immunization	Mother & litter No.	SV_{40} virus neutralizing antibody titer			SV_{40} tumor CF antibody titer		
		Mother	Litter		Mother	Litter	
		4 day	4 day*	1 mo*	4 day	4 day*	1 mo*
Live SV_{40} virus	1	Not done	1280	<20	Not done	<20	<10
				<20			<20
	2	>725	240	40	<10	<20	<10
				<20			<10
	3	20,480	970	40	<10	<20	<10
				<20			<10
				<20			<10
Control fluid	5	<5	<40	<20	<10	<20	<10
				<20			<20

* The sera tested were from offspring which had not been challenged with SV_{40} virus. The 4 day samples were from single animals and the 1 month samples were from 2 or 3 animals.

TABLE III. Tests for SV_{40} Virus Infection of Newborn Hamsters Whose Mothers Had Received Live SV_{40} Virus.

Maternal immunization	No. of litters	Virus isolation	Newborns not challenged with SV_{40} virus: Tumor incidence by age (days)						
			30	140	182	211	277	364	%
4 weekly i.p. doses of live SV_{40} virus	3	0/3*	0/7	0/7	0/6	0/6	0/5	0/1	0
4 weekly i.p. doses of control fluid	5	0/1†	0/14	0/13	0/12	0/10	0/8	0/8	0

* One 4-day-old animal out of each litter tested for virus.
† One 4-day-old animal from 1 litter tested for virus.

nor did they develop tumor during the 364 day observation period. This showed lack of *in utero* infection following maternal immunization with the virus.

Discussion. Experimental studies of virus-induced neoplasia in animals support the theory of virus etiology in human cancer and indicate that immunoprophylaxis might be of practical value in preventing the disease once viruses responsible for human neoplasia are identified. The present studies revealed passive transfer of SV_{40} antibody to the offspring and consequent protection of the progeny against tumor induction by SV_{40} virus administered early following birth. Immunization of the mothers with live SV_{40} virus prior to conception did not result in infection of the offspring as revealed by tests for presence of SV_{40} virus following delivery or by evolution of the tumor. The passive SV_{40} antibody was sharply reduced in amount within 1 month after birth. Tumor antigen CF antibody was not produced in the adult hamsters, indicating lack of significant neoplastic transformation by the agent.

Similar passive protection of the newborn against virus-induced neoplasia by maternal immunization has been shown for polyoma, Friend leukemia, adenovirus 12, and avian visceral lymphomatosis viruses under experimental or normal ecological conditions (5-12). Such procedure holds promise for effective vaccination against neoplasia in man caused by viruses.

Summary. Administration of live SV_{40} virus vaccine to adult female hamsters prior to conception afforded protection of the progeny against tumorigenesis by the homologous virus given shortly following birth. The mechanism appeared to be passive transfer of maternal antibody which prevented infection. There was no apparent infection of the fetus with SV_{40} virus *in utero* and there was no detectable CF antibody in the mothers or the progeny against SV_{40} tumor antigen. Maternal immunization holds promise as a means for preventing tumor induction in man caused by hypothetical viral neoplastic agents.

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Studies on Chemotaxis IX. Migration of Rabbit Leucocytes Through Filter Membranes.* (32538)

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Filter membranes are widely used in tissue culture experiments. In the diffusion chamber culture technique(1) the filters serve to separate the implanted cells from the host organism. On the other hand, the filters used in Boyden's technique for measuring chemotaxis *in vitro*(2) have to be permeable for

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