culinized when they were implanted into male larvae (*4*). More recently, Naisse (*5*) reported that the placement of a juvenile testis of the firefly, *Lampyris noctiluca,* into a young female larva resulted in an imaginal phenotype which was wholly masculinized. Likewise, the implantation of a juvenile ovary into a male larva resulted in a masculinization of the transplant. Apparently, a hormonal system controls sexual dimorphism in these instances. A seemingly different means controls divergent morphogenesis of genetically masculine gonads of *Aedes stimulans.*

At the beginning of postembryonic development of *Aedes stimulans,* gonadal discs of genotypic males have the capability of developing either into ovaries or into testes. The plasticity of the discs is retained during instars 1 and 2 when the rearing temperature is 26°C or higher (*1, 2*). If larvae are subsequently reared at 18°C for instars 3 and 4, the gonads of genotypic males are testes, whereas ovaries are formed if exposure to the high temperature is continued (*2*). In the following experiments, immature gonads were removed from genotypically male larvae which had been reared at 27°C for instars 1 and 2, and implanted into larvae in the third instar which had also been reared at 27°C during the first two instars.

The first experiments were designed to demonstrate the plasticity of the gonadal discs and to determine the means by which a juvenile gonad is masculinized. Each of the two gonadal discs of a genotypically male donor of *Aedes stimulans* was transplanted into a separate larva of the same species; either both of the host larvae were reared at 18°C, or one of the hosts was held at 27°C, and the other was kept at 18°C throughout development of the last two instars. A gonadal disc transplanted into a larva which was reared at low temperature always developed into a testis without regard to the sex of the host. Gonads belonging to the mosquito host developed normally. A gonadal disc from a genotypic male, when placed into a host larva reared at 27°C, always developed into an ovary irrespective of the genotype of its host. These experiments demonstrate (i) the plasticity of the gonadal discs, (ii) the effect of temperature on morphogenesis of imaginal organs, and (iii) that physiological factors originating from within the transplant are responsible for causing masculinity to be determined at 18°C. If maleness had been under humoral influence, then the gonadal disc when placed into a female larva reared at 18°C should not have developed into a testis, but invariably it did so develop.

Table 1. Differentiation of the two gonadal discs from each genotypically male donor (*Aedes stimulans*) transplanted into separate host larvae of *Aedes vexans*, one of which was subsequently reared at 27.0° ± 0.1°C and the other at 18.0°C ± 0.1°C for instars 3 and 4.

No. of individual gonads transplanted*	Genotype of host	Rearing temperature for instars 3 and 4 (°C)	Phenotype of imaginal gonads	
			Host	Donor
6	♂	27	Testes	Ovary
6	♂	18	Testes	Testis
4	♀	27	Ovaries	Ovary
4	♀	18	Ovaries	Testis
4	♀	27	Ovaries	Ovary
4	♂	18	Testes	Testis
6	♂	27	Testes	Ovary
6	♀	18	Ovaries	Testis

* Each of the two gonadal discs of a donor was placed into a separate host.

The first experiments do not demonstrate the process by which the genetic male transplant becomes feminized at high temperatures. The gonads of both genotypic males and genotypic females of *Aedes stimulans* are wholly feminized at 27°C. Since the host's gonads are always ovaries at 27°C, it is not possible to ascertain whether the implanted disc became feminized independently of factors in the hemocoel. To determine whether femininity is under the influence of physiological factors extrinsic to or intrinsic in each imaginal disc, an exotic species, *Aedes vexans,* was used as a host, because normal males of this species are obtained even at 35°C. A plastic gonadal disc of *A. stimulans* implanted into a male or female larva of *A. vexans* which was reared at 27°C for instars 3 and 4 developed into an ovary (Table 1). The second disc from the same donor implanted into a female or male larva of *Aedes vexans* which was reared at 18°C for the last two instars developed into a testis (Table 1). Feminization is evidently due to physiological factors wholly intrinsic within each imaginal disc. If femininity was under humoral influence, then the disc placed into a male host at 27°C should not have developed into an ovary.

These experiments do not wholly exclude the possibility of a feminizing hormone. It is possible that at 27°C, but not at 18°C, a feminine hormone is secreted in males of *Aedes vexans,* but because of a difference of sensitivity of tissues between the two species, only the implant responds. This alternative interpretation, however, seems highly unlikely. Gonadal discs from genotypically female larvae of *Aedes stimulans* were transplanted into larvae treated in the manner described in the preceding paragraphs. These organs, as expected, developed into ovaries irrespective of the sex of the host or the temperature of the rearing medium.

JOHN F. ANDERSON*
WILLIAM R. HORSFALL
Department of Entomology,
University of Illinois, Urbana

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* Present address: Connecticut Agricultural Experiment Station, New Haven.

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Tumor and Virus Antigens of Simian Virus 40: Differential Inhibition of Synthesis by Cytosine Arabinoside

Abstract. *Cells infected with the papovavirus SV40 not only synthesize viral antigen but also synthesize the specific nonviral antigen found in SV40-induced tumors. In the presence of the DNA antagonist cytosine arabinoside, infected cells fail to make viral antigen but still synthesize the tumor antigen. Iododeoxyuridine does not inhibit the synthesis either of tumor or of virus antigen but does prevent the development of infectious virus.*

The development of methods for detecting the tumor antigen induced by simian papovavirus 40 (SV40) both in cells transformed by the virus (*1, 2*) and during the early stages of the replication of the virus in cytolytic systems (*3, 4*) has resulted in an accumulation of data on the tumor and virus antigens in differing experimental systems. During studies of the effects of DNA in-

Table 1. Synthesis of SV40 tumor and viral antigens in monkey kidney cells exposed to DNA antagonists. TA, SV40 tumor antigen; VA, SV40 virus antigen; EM, electron microscopy; IUDR, 5-iodo-2-deoxyuridine; CA, cytosine arabinoside; PI, post inoculation.

Inhibitor	Concn. μg/ml	PI (hr)	Complement fixation*		Immunofluorescence†		EM virus particles
			TA	VA	TA	VA	
CA	10	24	32	<2	25	0	Absent
CA	10	48	64	<2	50	0	Absent
IUDR	50	24	32	4	25	<0.1	Rare
IUDR	50	48	64	256	25	25	Present
None		24	16	32	25	5	Present
None		48	64	1024	50	75	Present

* Titers given are reciprocal of highest dilution of antigen yielding less than 50 percent hemolysis, against 1:20 hamster serum (from a tumor-bearing animal) for measuring the tumor antigen, and against 1:40 monkey serum (from an SV40-infected animal) for measuring the virus antigen. † Numbers represent approximate percentage of cells containing respective antigens.

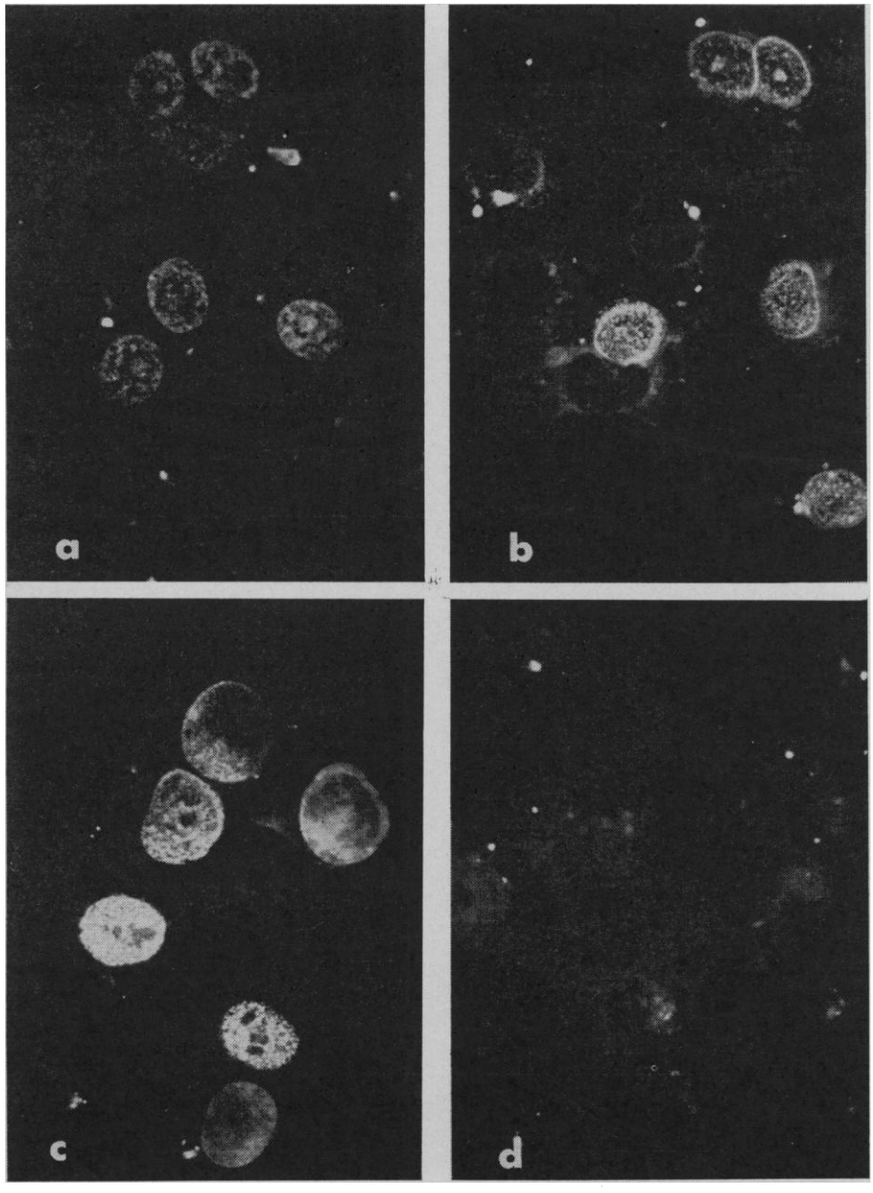

Fig. 1. Immunofluorescence of monkey kidney cell cultures inoculated with SV40. About × 370. *a*, Reacted 24 hours after inoculation with hamster antiserum for detecting SV40 tumor antigen. No inhibitor present. *b*, Reacted 24 hours after inoculation with hamster antiserum for detecting SV40 tumor antigen. Maintained in presence of 10 μg of cystosine arabinoside per milliliter. *c*, Reacted 48 hours after inoculation with monkey antiserum for detecting SV40 viral antigen. No inhibitor present. *d*, Reacted 48 hours after inoculation with monkey antiserum for detecting SV40 viral antigen. Maintained in presence of 10 μg of cytosine arabinoside per milliliter. White areas represent intranuclear antigens.

hibitors on the synthesis of both SV40 tumor and virus antigens, it was noted that cytosine arabinoside inhibits synthesis of viral but not tumor antigens, whereas iododeoxyuridine inhibits neither tumor nor viral antigen.

Primary kidney cell cultures from African green monkeys *(Cercopithecus aethiops)* were grown in Melnick's medium (*5*). The cell cultures were inoculated with a suspension of SV40 to provide ten plaque-forming units (PFU) per cell; adsorption took place for 1 hour at 37°C. The cells were then flooded with fresh medium containing the inhibitors (*6*) in the concentrations indicated below. The inoculated cultures were incubated for 24 to 48 hours at 37°C in an atmosphere of 5 percent CO_2. For detection of tumor and virus antigens by the immunofluorescence technique, cells were grown on coverglasses (15 mm). Tumor antigen was detected by reacting the cells with serum from hamsters bearing SV40 tumors and then with rabbit globulin prepared against serum globulin from normal hamsters; the rabbit globulin had been labeled with fluorescein isothiocyanate. The virus antigens were detected with a similarly labeled monkey serum globulin prepared against SV40 virus. The micro complement-fixation tests were carried out with antigens prepared from 16-ounce (500 ml) bottle cultures. Serial (twofold) dilutions of the antigens were reacted with a standard concentration of antiserum (*2, 3, 7*).

Twenty-four hours after inoculation of the cultures with SV40, tumor antigen was readily detected, by complement fixation, in control cultures and in cultures to which cytosine arabinoside (10 μg/ml) or iododeoxyuridine (50 μg/ml) had been added (Table 1). Immunofluorescence revealed that 25 percent of the cells were elaborating the tumor antigen in the nucleus at this time (Table 1; Fig. 1, *a* and *b*). By the 48th hour, 25 to 50 percent of the cells were synthesizing the antigen. The same results were obtained with the complement-fixation tests.

In the infected drug-free cultures, viral antigen was detected by complement fixation at 24 hours; the concentration rose sharply between 24 and 48 hours after inoculation (Table 1). Only 5 percent of the cells were synthesizing these antigens at 24 hours but 75 percent of the cells were elaborating them 48 hours after inoculation (Table 1; Fig. 1*c*). In the presence of cytosine arabinoside (10 μg/ml) the cells did

not synthesize virus antigen as judged by either complement-fixation or immunofluorescence techniques (Table 1; Fig. 1*d*). However, iododeoxyuridine (50 μg/ml) did not halt synthesis of viral antigen, although the titer of the antigen was lower than that of the controls (Table 1). These results were confirmed in tests with other concentrations of the inhibitors. Additional confirmation was obtained by examining the antigens used in the complement-fixation test by electron microscopy after staining with uranyl acetate (*8*). No virus particles were observed in preparations from cultures treated with cytosine arabinoside, although virus particles (*8*) were readily observed in antigens prepared from control cultures and from cultures maintained in the presence of iododeoxyuridine.

A preparation of adenovirus 7, shown to induce SV40 tumor but not virus antigen (*9*), was also tested for SV40 genome activity in the presence of cytosine arabinoside and iododeoxyuridine. The SV40 tumor antigen was synthesized in the presence of both inhibitors in the same way that it was produced in cells inoculated with SV40 itself.

Both iododeoxyuridine and cytosine arabinoside inhibit the replication of the DNA-containing vaccinia and herpes viruses (*6*, *10*) but cytosine arabinoside appeared to be more potent than iododeoxyuridine as an inhibitor of the replication of herpes viruses. Like fluorouracil (*7*), iododeoxyuridine also prevents replication of infectious SV40 (*11*). Our observations suggest that the DNA antagonists tested differ in the manner in which they inhibit the replication of DNA viruses. Apparently the portion of the genome coding for the synthesis of SV40 tumor antigen is less sensitive to the action of cytosine arabinoside than the genome responsible for coding for virus antigen. The inhibition of the synthesis of viral antigen in a system allowing the elaboration of SV40 tumor antigen will greatly aid both the separation and the purification of the tumor antigen.

FRED RAPP
JOSEPH L. MELNICK
TSUNEHIRO KITAHARA
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

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Adrenal Response to Fighting in Mice: Separation of Physical and Psychological Causes

Abstract. *The concentration of unbound corticosterone in mice exposed to the presence of a trained fighter is much greater if the mice have previously experienced physical defeat than if they have not. There is little difference in the concentration of the hormone between mice placed in the presence of a fighter, given a background of physical defeat, and mice actually attacked and defeated. Two possible categories of stimuli which could be responsible for hyperactivity of the adrenal cortex following defeat by another mouse are psychological and physical (for example, bite wounds); the former is apparently by far the more important under the conditions of these experiments.*

Crowding of mice and its resulting "social stress" (*1*) have become important variables in many areas of biological experimentation. Among those effects attributed to social stress are decreased resistance to disease (*2*) and endoparasitism (*3*), lowered reproductive performance (*4*), and altered behavior of offspring of crowded mothers (*5*). Such varied effects are thought to be, directly or indirectly, due to increased activity along the adenohypophyseal-adrenocortical axis.

Many investigators (*6*) have thought the actual stimuli which elicit the adrenal and pituitary responses among crowded mice to be primarily psychological rather than physical (for example, systemic effects due to bite wounds), but experimental evidence is inconclusive. It is known that fighting among males contributes heavily to the degree of adrenal response occurring among grouped mice. However, the results of two studies concerned with crowding were somewhat conflicting with respect to the relative importance of wounding as opposed to sociopsychological stimulation (*7*). We have therefore investigated adrenal responses (unbound corticosterone in the plasma) in mice placed in the presence of a trained fighter. Mice which had previously experienced physical defeat by a fighter showed a much greater adrenal response to the fighter's presence than did mice not having experienced defeat, thus demonstrating a psychological component and yielding a basis for comparing the relative roles of psychological and physical stimulation.

All mice (180 C57BL/6J) were weaned at 21 to 28 days and reared in isolation (in stainless steel boxes, 15 by 30 by 15 cm) until used in the experiment at 85 to 95 days of age. All mice remained in isolation during the 6- to 9-day experimental period except for 15 minutes each day. The basic experimental treatment consisted of placing a mouse in the home cage of a trained fighter of the same strain; but the subject was physically separated from the fighter by a removable partition consisting of two layers of 0.6-cm wire mesh mounted on each side of a wooden frame. Procedures following such initial separation from the fighter varied among three experimental groups of 60 mice each; in group 1 the partition was always removed after 10 minutes, allowing the fighter to attack the subject for 5 minutes; in group 2 the partition was removed as in group 1 for the first 5 days, but remained in place for the entire 15-minute period after day 5; and in group 3 the partition was never removed. Twenty mice from each of the three groups were killed for blood collection on each of days 6, 7, and 9 of the experiment. Two of the groups, therefore, were physically exposed to the fighter on each of 5 days. In one of these groups (group 1) physical exposure continued on each of days 6 to 9 when blood was collected, and the other (group 2) was exposed to only the presence of a fighter during the period of blood collection. Group 3 served as a control group. Physical exposure of an untrained mouse to a trained fighter invariably results in almost instantaneous attack by the fighter, defeat of the un-