High concentration MI of parabasal cells on above dates (Fig. I) resembles horomonal withdrawal pattern, which could be caused by the four different corpora luteal complex. This is further supported by our observation that in 6 animals which, in spite of several matings, never became pregnant, and an autopsy showed small ovaries with no maturing follicles. They also had a high count of P 36%.

Summary. Studies on the effects of estrogen–progestrone levels on the MI of the squamous epithelium of vagina of pregnant rats were made. We observed fluctuations in MI for different periods of gestation as well as variations in cytological criteria which occurred especially on days 7 and 14. The MI shifts and marked alterations of the cytological criteria coincides on the same gestational days.

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Biological Separation of Adenovirus T and V Antigen Synthesis in KB Cells* (32762)

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Sero-epidemiological studies of the relationship of the oncogenic DNA viruses to human cancer are plausible based on the occurrence of virus specific nonvirion T antigens in tumor cells and the occurrence of specific antibody in animals bearing such tumors (1).

We have explored several methods of producing T antigens for the human adenoviruses with the aim of devising a single procedure which would prove applicable to both non-oncogenic and oncogenic viruses. The desired result is a T antigen preparation, free of viral structural antigens, of sufficient titer to test sera obtained from carefully matched cancer patients and controls. While there are multiple possible sources of antigen and a variety of possible approaches to this problem, this paper will be restricted to experiments involving the lytic interaction of adenoviruses in KB cells.

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Materials and Methods. Virus. Adenovirus types 7 (Gomen) and 12 (Huie), obtained from the American Type Culture Collection and the Trailer Facility of the Laboratory of Viral Diseases, National Institutes of Health, respectively, were passed several times in human embryonic kidney (HEK) cells prior to preparation of standard pools in this tissue. Pools at the third to fifth and second to fourth passages of adenoviruses 7 and 12, respectively, with infectivity titers in HEK cells (2) of $10^{7.5}$ to $10^{8.5}$ and $10^{7.2}$ to $10^{7.7}$ $TCID_{50}$ per ml, were used in these studies. Virus pools and all T antigen preparations were tested and found negative for adeno-associated viral (AAV) antigens (3).

Complement fixation (CF) tests. The microtiter procedure using 1.8 exact units of

C′ and overnight fixation at 5°C (4) was used.

Ultraviolet (UV) irradiation. A General Electric germicidal lamp (no. G15T8, 15 W) was employed which was adjusted to deliver 3100 ergs/cm^2 through a distance of 20 cm to the surface of a 60 × 15-mm petri dish. Virus was irradiated in 3 ml quantities on a rotating shaker (80 rotations/min).

Reagents. The CF tests for adenovirus hexon antigen (5) initially employed a standard adenovirus human convalescent serum; later a guinea pig antiserum prepared against purified type 7 hexon antigen following the procedure of Norrby (6) became available and also was used. Certain preparations were also assayed with guinea pig antibody prepared against the type 7 penton antigens with the purified type 7 complete hemagglutinin (6). Specific T antisera for both adenoviruses 7 and 12 were prepared according to our published procedures (7).

General procedures. The KB cells, (obtained originally from Dr. H. S. Ginsberg), at or near confluence in "milk dilution" bottles were allowed to adsorb virus for 1 hour at 37°C. Input virus multiplicities, based upon prior titration in HEK tube cultures, were 15–31 for type 7 and 0.2–1.2 for type 12. The inoculum was then removed. The cell sheet was washed with Earle's balanced salt solution to remove the residual inoculum and maintenance medium (Eagle's Basal Medium supplemented with glutamine, penicillin, streptomycin, and 5% newborn "agamma" calf serum) added. Cells were harvested by scraping them into the medium and, following centrifugation, the medium was discarded. The cell pellet was washed in salt solution, recentrifuged, and frozen. For CF testing cell pellets were thawed in Veronal–buffered saline to a 20% v:v suspension and used without further clarification.

Thermal separation. A 1-hour absorption at 37°C was always employed followed by variable further incubations at 37°C before transfer to low temperature. In early experiments, room temperature was used without careful control. More recently, a Precision Scientific B. O. D. incubator set at 25–26°C has been used for more accurate temperature control.

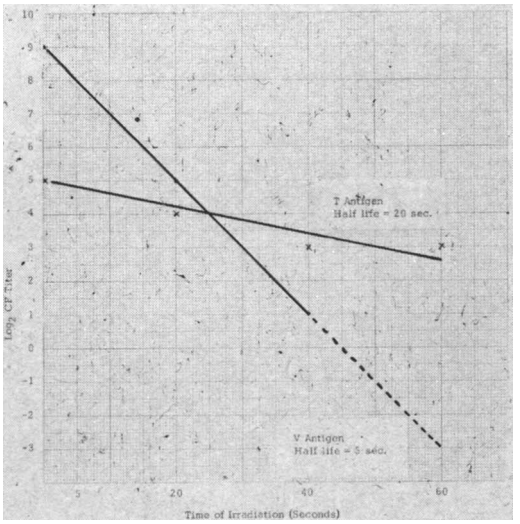

FIG. 1. Ultraviolet inactivation of type 7 T and V antigens. Three ml of virus was irradiated for various times under the conditions of lamp distance and emitted energy given in the text. The KB cells were infected with a multiplicity of approximately 30, based on the original titration of unirradiated virus in HEK cells. The CF tests were carried out with human convalescent serum for the V, and tumored hamster serum for T antigen. All samples were harvested 24-hours postinfection and tested as frozen–thawed 20% v:v suspensions. The data show a fourfold difference in inactivation rates between the T and V antigens.

Results. A. DNA Inhibitors. The major problem to be overcome is the large amount of structural antigen synthesized in KB cells. With viral inputs exceeding 25 (type 7), titers of 1:512 to 1:1024 are routine by 48-hours postinfection. At this time T antigen titers are generally maximal but 20-fold or more lower than V titers. In the particular KB cell line used, cytosine arabinoside at 100 μgm/ml of medium, even freshly added at 12-hour intervals, did not alter V antigen production; however, 5-fluorodeoxyuridine (FUDR) generally reduced V titers by 90–95% with minimal effect on T titers as previously reported for this cell line (8). This generally resulted in equivalent T and V titers, e.g., 1:16–1:32 for type 7, which although theoretically satisfactory, did not achieve the desired practical result.

B. Inactivation by UV. An approximately 4-fold difference in inactivation rates of the

TABLE I. Separation of Rates of Synthesis of Adenovirus 7 T and V Antigens in KB Cells by Room Temperature Incubation.

Incubation time (hours)		CF titers	
37°C	Room temp.	T	V (hexon)
4	24	2[a]	<1
4	48	4	<1
4	72	32	<1
4	96	16	32
8	24	4	1
8	48	16	8
8	72	16	16
8	96	32	64
24	None	8	64
48	None	16	1024

[a] Reciprocal of titer; input multiplicity = 31.

ability to induce T and V antigens was found for both adenovirus types 7 (Fig. 1) and 12, thus providing a possible method of achieving the desired result. Extensive studies with this system revealed complexities, mainly DNA repair, which limited incubation times to less than 36 hours before viral antigen synthesis was restored. This limited severely the use of this procedure to attain our particular goal since T antigen titers were relatively low at this time. In those cell systems in which the original ratio between T and V antigens is more favorable, the UV irradiation procedure should be of great value.

C. Thermal separation. A procedure has been described by Kitahara and Melnick (9) for the thermal separation of T and viral antigens of SV_{40}. This procedure has been applied to both adenovirus types 7 and 12 in KB cells with the general result that nearly complete (99.5%) inhibition of viral antigen synthesis can be achieved with minimal loss of T antigen titer. The data in Table I are representative of three separate experiments; however, in multiple experiments low levels of V antigen were detected (1:1–1:4) but were always 8-fold lower than T titers. We have found it necessary to use the KB cells at a specified passage range (10–30 passages in our laboratory), since for as yet an unknown reason(s), harvests at high passage levels (>30) were frequently anticomplementary and titers were significantly reduced. This was found to be independent of PPLO contamination. Under controlled condition, combination of the thermal procedure with FUDR in the medium should provide the practical approach for large-scale T antigen preparations.

To achieve reasonable T titers within 48- to 72-hours postinfection, a short incubation at 37°C was found necessary; the limit to regularly avoid detectable V synthesis was 4 hours, while 6- or 8-hour periods were usually too long (Table I). The maximum period of incubation at the usual low temperature employed (24–26°C) with minimal V antigen formation was 72–96 hours. V antigen levels reaching normal values were obtained within 24 hours if cultures were returned to 37°C. This synthesis was inhibited by FUDR and thus is assumed to be dependent on DNA synthesis.

Similar results were obtained with both types 7 and 12 and indicated the general utility of this procedure.

Discussion. Three biological manipulations of the human adenovirus KB cell interaction were explored, the purpose being the production of T antigens, free of virion antigens, for sero-epidemiological studies of human cancer. Our results indicate that the thermal separation procedure, similar to that described previously for SV_{40} (9) is extremely effective in dissociating T and V antigen synthesis of the adenoviruses. The additional use of FUDR in the medium should serve to completely suppress the low level viral antigen titers obtained in some of our replicate experiments. With regard to the non-oncogenic adenoviruses, it seems clear from fluorescent antibody studies (10–12) and our own unpublished CF data that antigens reactive with oncogenic virus T antibody are formed in lytic interactions of these viruses. We tentatively assume that control of multiplicity of infection and other factors will produce equivalent T and V separations for the non-oncogenic viruses as well.

The UV inactivation data are in essential agreement with the recent report of Gilead and Ginsberg (13) showing a 4-fold difference in inactivation of *infectivity* and T antigen synthesis for type 12. A similar relative rate of inactivation was found previously for SV_{40} T antigen and infectivity (14). It is assumed

that V antigen synthesis represents a terminal or near terminal synthetic step in virus replication and thus the close agreement of V antigen and infectious virus inactivation is to be expected.

We have restricted our discussion to biological methods of effecting differential synthesis of T and V antigens. These methods clearly provide a valuable initial procedure for T antigen purification studies since V antigens constitute a substantial portion of the total protein of adenovirus infected KB cells. Essentially similar results have been found by Rhim and associates.[1]

Summary. Adenovirus T and virion antigen syntheses are readily separable by use of DNA antagonists, e.g., FUDR, thermal separation, and UV irradiation of virus. Of these, the thermal separation procedure was most effective, and coupled with the use of FUDR, should provide a relatively simple procedure for large-scale preparations of T antigens for sero-epidemiological surveys.

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Temperature Dependence of the Synthesis of Adenovirus Tumor and Viral Antigens* (32763)

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Specific complement-fixing (CF)(1) and fluorescent antibody T (tumor) antigens (2) have been described in adenovirus-induced hamster tumors or in adenovirus-transformed hamster or rat cells (1–5). Synthesis of the T antigen (6) also occurs in cells infected with adenoviruses; however, the antigen appears early in the cytolytic cycle, before the appearance of the virus itself (2,7,8). The adenoviruses have recently been divided into three subgroups, A, B, and C, based upon their oncogenic properties in newborn hamsters, their T antigens, and their DNA base compositions (9–11).

Studies designed to produce virion-free T antigen by attempting to block adenovirus virion synthesis using 5-FUDR (8,12) and cytosine arabinoside (13) have been moderately successful; however, it has been suggested (10) that the production of working amounts of adenovirus T antigen free of structural virion antigens may prove to be more difficult than the production of virion-free SV-40 T antigens (14–16). Recent reports described the selective production of

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