tidiuresis and greatly enhance hyperglycemic activity.

Summary. An analogue of chlorthalidone (C3/76) has been studied in an attempt to obtain a long acting hyperglycemic agent. In rats C3/76 (administered i.p.) caused a dose-related hyperglycemia which lasted longer than that of diazoxide and was reversed by insulin. The drug was effective in both fed and fasted animals and the hyperglycemia was accompanied by a reduced urine output. In the dog, i.v. administration of C3/76 caused hyperglycemia, a fall in blood pressure, an inhibition of insulin secretion, a rise in serum free fatty acid levels, and no significant change in serum catecholamine levels. In *in vitro* studies with isolated rabbit pancreas, C3/76 reduced insulin secretion, while chlorthalidone had no effect. It can therefore be concluded, that C3/76 produces hyperglycemia by inhibition of insulin secretion similar to one of the reported modes of action of diazoxide. It has similar side effects, hypotension and antidiuresis, even though its chemical structure is quite dissimilar to that of diazoxide.

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Immunofluorescent Study on Early Virus-Cell Interaction in Shope Papilloma *in Vitro* System* (33073)

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Appearance of a new antigen, now widely referred to as T antigen, in cells infected with SV40, polyoma and adenovirus has been well established (1–3). The role of this new virus-related antigenic substance in lytic infection or in the neoplastic transformation has not been well understood as yet. Mainly due to the fact that a suitable *in vitro* experimental system was not available, few studies have been carried out on the "early events" of the virus-cell interaction in the Shope papilloma system. In a previous communication (4), we have presented some preliminary observations on the appearance of T antigen-like immunofluorescence in the nuclei of cells of embryonic skin culture approximately 20 hours after their exposure to the Shope papilloma virus (SPV). The present paper describes the results of more detailed findings suggesting that such a new antigen, which could be clearly distinguished from the viral structural antigen, does appear in the early

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TABLE I. Immunoassay of Antipapilloma and Anti-SPV Antisera against SPV Preparation.

Antigen	Assay method	Antibody titer[a] in		
		Antipapilloma antiserum	Anti-SPV antiserum	Normal serum
SPV ($10\ ID_{100}$)	Neutralization	< 10	1280	< 10
SPV ($10\ ID_{100}$)	Complement fixation	< 5	200	< 5

[a] Titer/ml of antiserum.

stage of infection of the *in vitro* cultured rabbit cells after being exposed to SPV.

Materials and Methods. Cells. Epithelial tissue from embryos of domestic rabbits of about 2 weeks in age was minced with surgical scissors, treated with 0.25% trypsin, washed with YLE medium (Earle balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolyzate) and was cultured in TD40 flasks with Eagle's medi-

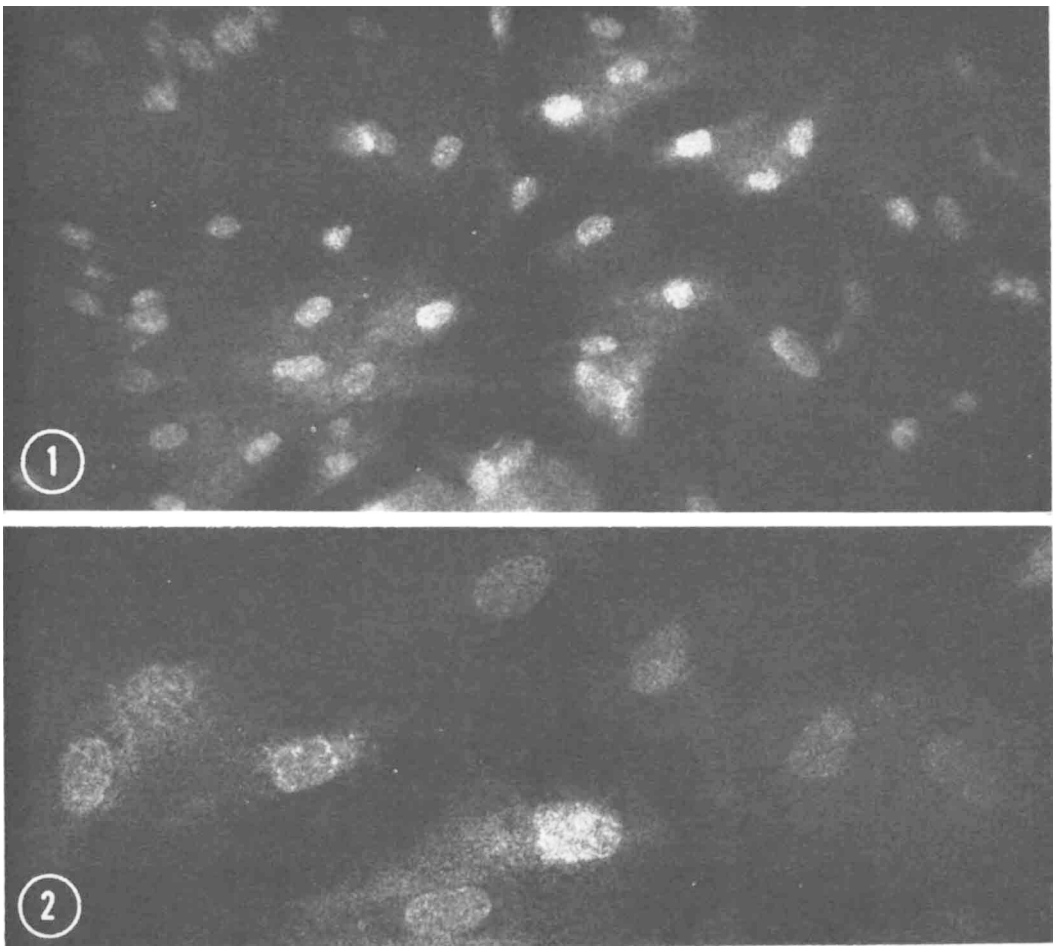
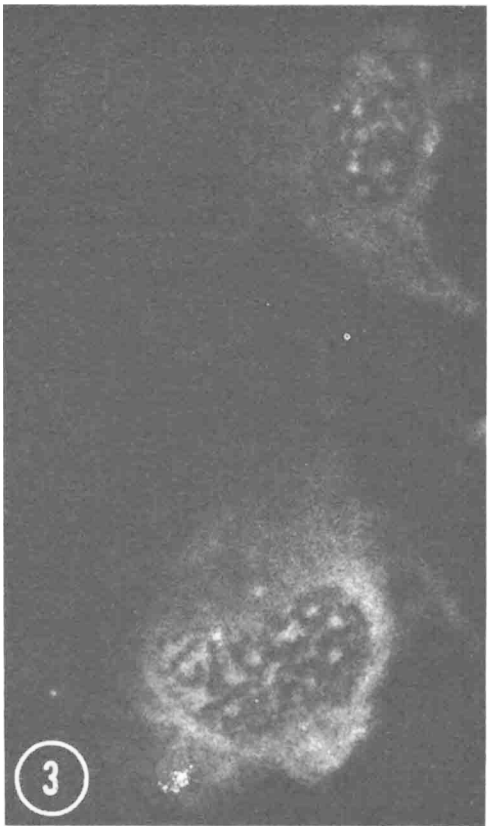


FIG. 1. Fluorescent photomicrograph of SPV-infected embryonic skin culture of domestic rabbit; 11 hours after infection; reacted with anti-papilloma antiserum (direct method). The majority of cells show intense specific fluorescence in the nucleus. ×100.

FIG. 2. Higher magnification of the same preparation as shown in Fig. 1. ×200.



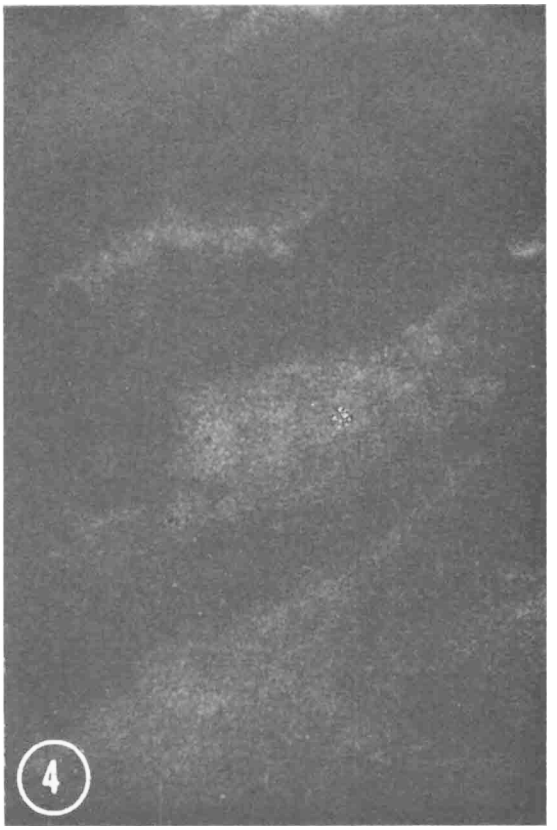


FIG. 3. SPV-infected cells 21 hours after infection. Note the granular fluorescence in the nuclei of two cells in the field. ×400.

FIG. 4. Noninfected control cells, 21 hours in culture, stained with antipapilloma antiserum. No specific fluorescence is seen. ×400.

um, supplemented with 20% horse serum. The culture was carried out at 37°C in a stationary position. In the initial stage of the study, the trypsinized primary culture cells were seeded on coverslips to obtain the secondary culture and these were used as the cells to be exposed to SPV. Later, a cell-line was established from the primary culture and the coverslip culture was made from these cells whenever necessary.

Virus. The source of SPV employed in the present study was glycerinated tissue of cottontail rabbit. The material was a generous gift of Dr. C. A. Evans, Department of Microbiology, University of Washington, Seattle. A 10% tissue extract was prepared by our standard procedure (5) and was preserved at —70°C until the time of use. This stock SPV preparation was diluted 2 or 10 times with phosphate buffered saline (PBS) for use as an inoculum.

Antisera. (a) Antipapilloma antiserum was prepared by inoculating the rabbit with its autologous SPV-induced papilloma tissue repeatedly as previously described (7). (b) Anti-SPV antiserum was prepared by immunizing domestic rabbit with partially purified SPV preparation as the antigen. The antibody titer in these two antisera tested by neutralization and complement fixation are listed in Table I.

Immunofluorescence. For the attempts to detect T antigen in the SPV-infected embryonic rabbit skin culture, the direct fluorescent test was employed with fluorescein isothiocyanate (FITC)-conjugated antipapilloma antisera. The sera were absorbed with acetone powder of newborn rabbits before use to

eliminate the nonspecific antibody against normal component(s) of the rabbit tissue possibly present in the sera. The detection of SPV antigen was carried out by an indirect method. The details of the procedure employed have also appeared in a previous report (4). The cultured cells were inoculated with 0.3 ml of SPV per coverslip and incubated at 37°C for 30 min. After subsequent washing with PBS, 1.0 ml of culture medium was added and cultivation was continued. The samples were taken both from SPV-infected and noninfected control cultures at 5–6, 10–11, 20–22, and 30–34 hours, washed twice with PBS, air-dried and fixed in carbon tetrachloride for 15 min at room temperature. The procedure for the immunofluorescent staining has been described in detail in a previous paper (4).

Results. In samples taken 6 hours after the exposure of the cells to SPV, approximately 10–20% of the cells were seen with granular fluorescence in the nuclei. At 10 hours, about 90% of the cells exhibited fine granular intranuclear fluorescence (Figs. 1 and 2). The nuclear membrane of these cells was also clearly stained. At 20–22 hours, the fluorescent granules in the nuclei tended to fuse together. Subsequently, the number of granules per nucleus decreased but the size of each granule increased and became more conspicuous (Fig. 3). In samples of cultured cells taken at 30–34 hours after the exposure to SPV, the "fusion" of the fluorescent granules proceeded and formed a relatively large mass in the nucleus (Fig. 5). The localization of these fluorescent sites was suggestive of morphological correspondence to the site of the nucleolus of the cells. However, definite correlation was not feasible.

Noninfected control cultures corresponding to the experimental sets at each stage were treated by a similar procedure with anti-papilloma antiserum. None of the cells showed specific fluorescence neither in the nucleus nor in the cytoplasm (Fig. 4).

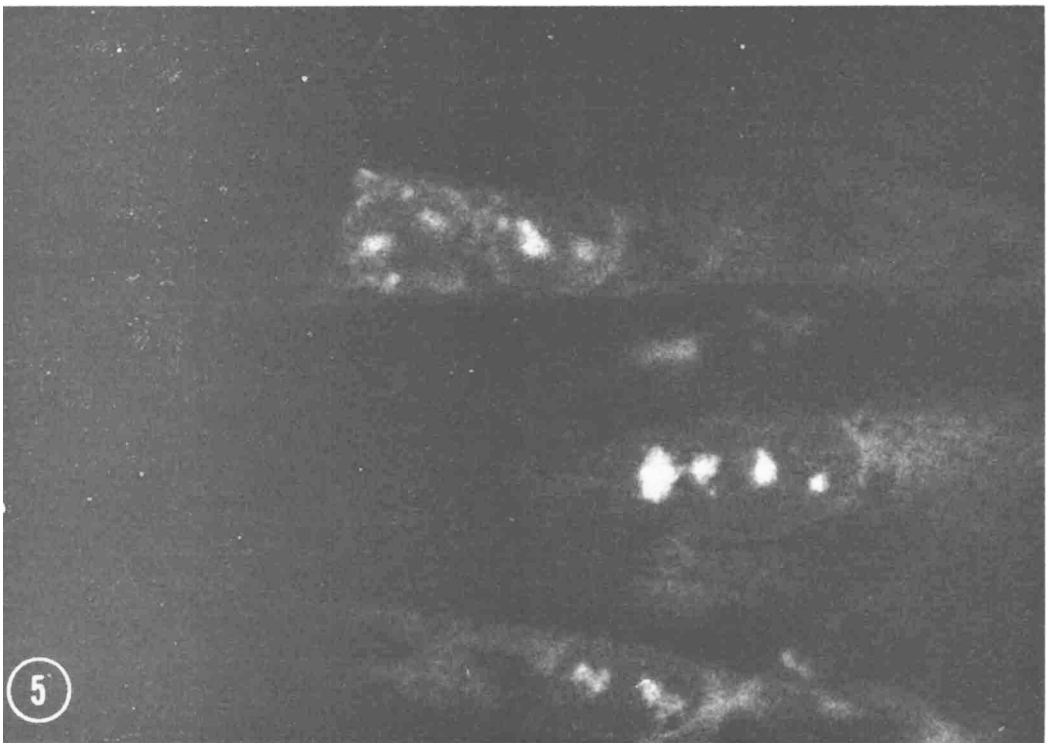


FIG. 5. SPV-infected cells 34 hours after infection. The fluorescent granules tend to "fuse" and form a dot-like figure in the nucleus. ×400.

The samples of infected cells from all these stages were also stained with anti-SPV antiserum. No significant amount of SPV-antigen was detectable in cells of any of the samples examined.

Discussion and Conclusion. Embryonic skin culture of domestic rabbit was infected with Shope papilloma virus *in vitro* and the early phase of virus-cell interaction was studied sequentially up to 30-hours post infection by the immunofluorescence technique. The cells of infected cultures showed a specific fluorescent reaction in the nucleus when stained with antipapilloma antisera conjugated with FITC. The ratio of positively stained cells was about 20% of the whole cell population in cultures 5–6 hours after infection with the virus and it reached a maximum of approximately 90% at around 11 hours. The morphological pattern of the intranuclear fluorescent staining was reminiscent of the reaction of T antigen reported in the SV40-infected hamster cells (6).

Kreider *et al.* (7) have recently reported their findings on the persistence of Shope papilloma virus absorbed onto the cell surface of cells of *in vitro* cultured embryonic skin of rabbits. The viral antigen was detectable in cultured cells even after 6-days post-infection by immunofluorescence. The fluorescent granules were seen randomly distributed all over the cell in most of the cases.

The specific immunofluorescence in the SPV-infected embryonic cells as shown in the present study was clearly associated with the nucleus and the positive staining reaction was only induced by the antipapilloma antiserum. Experiments with the anti-SPV antiserum yielded only negative results. From these findings, it seems plausible to conclude that the antigen demonstrated in the nucleus of the SPV-infected rabbit cells by immunofluorescence possibly meets at least a part of the criteria of T antigen. Studies are being carried out to assess the problem from different approaches such as complement fixation test between the cellular extract of the infected cell culture and the antipapilloma and anti-SPV antisera.

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Specificity in Protection against Lethality and Testicular Toxicity from Cadmium* (33074)

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Cadmium salts, administered subcutaneously, cause selective destruction of the testis (1–3). Selenium compounds are very effective in blocking this reaction (4,5) and we questioned whether they might also diminish the generalized acute toxicity of larger amounts of cadmium. The following experiments were undertaken in male mice to determine if doses of cadmium, normally fatal,

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