Propagation of Fibroma Virus in Tissue Cultures of Cottontail Testes. (22598)

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This paper describes propagation of Shope fibroma virus through 14 successive passages in cultures of cottontail (*Sylvilagus* sp.) testes and through a few passages in similar cultures of testes from domestic rabbits. References to propagation of fully active fibroma virus *in vitro* have not been encountered(1).

Materials and methods. Virus. Fibroma virus employed was one originally isolated from a wild cottontail(2). Two lines of this virus were propagated *in vitro*. One line, designated A, had been passed intradermally in cottontails, and another, B, was the same virus after 2 intravenous passages, also in cottontails. Propagation in tissue cultures was initiated with 10% suspensions of skin fibromas. *Tissue cultures.* Although some mature cottontail testes were used in later passages, testes from immature or from non-breeding animals were selected by preference. Testes of domestic rabbits were uniformly selected from immature animals. The tissues were minced with scissors prior to fixation in roller tubes by means of chicken plasma clot. Cultures were fed maintenance solution(3) plus 20% horse serum initially and 10% horse serum in 4 to 8 days when cellular growth was well started and the cultures were inoculated. Each tube contained 2 ml of supernatant fluid. At intervals of 3 to 10 days (usually 4 or 5) transfers were made by the inoculation of tubes with 0.2 ml of fluid from the previous passage. Monolayer monkey kidney cells(4) and explants of human foreskin were cultivated under similar conditions. *Tests for virus.* Presence and amounts of virus in supernatant fluids were determined by intradermal inoculation of domestic rabbits by methods previously described(2). Maintenance fluid containing 10% horse serum was used as a diluent in titrations and in storage of virus. *Animals.* Albino rabbits were procured from local dealers, and cottontails were purchased from dealers in Wyoming and Kansas or were trapped locally. *Histology.* For histological examinations testis tissue grown on cover slips was fixed in Bouin's solution and stained with hematoxylin and eosin.

Results. Cottontail testes cultures. The 14 passages through which lines A and B of fibroma virus were carried represented 18 and 20 tenfold dilutions respectively of the original fibroma suspensions. Since these original suspensions titrated only 10^{-5}, a considerable increase of virus occurred. Multiplication of fibroma virus was evident within individual passages. Fig. 1 demonstrates such results of skin tests in a domestic rabbit. Third passage of line A, with virus barely demonstrable in undiluted supernatant fluids obtained 3 days after inoculation, had a virus titer of 10^{-2} at 7 days and of 10^{-3} at 13 days after inoculations. In sixth passage, there was a rise of from 10^{-3} at 13 days to 10^{-4} between 4 and 8 days after inoculation, fluids having been changed and titrated at each interval. In all passages, with either line of virus, 10^{-4} was the maximum virus titer obtained. Titers usually declined at later intervals when the tissue culture cells were degenerating. Such degeneration, after a variable number of weeks, was equally demonstrable in uninoculated control cultures.

Cultures of domestic rabbit testes. The only success in propagation of fibroma virus in cultures of testes from domestic rabbits was with line A virus, which was recoverable, although barely so, from fourth passage supernatant fluid. There was suggestive evidence of virus multiplication in the first 2 passages. Thus, in the second passage, supernatant fluid had a virus titer of 10^{-2} 4 days after inocula-

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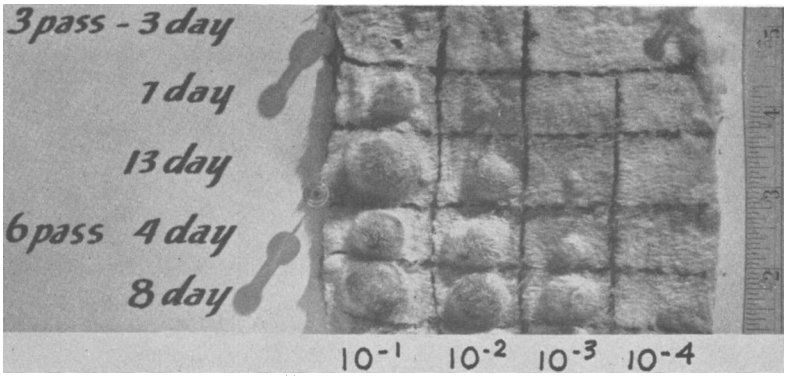

FIG. 1. Skin of a domestic rabbit, showing results of titrations of fibroma virus as it occurred in supernatant fluids of various tissue culture passages. Photograph taken 8 days after skin inoculations were made.

tion. After 2 complete changes of fluid in the course of 6 days, the supernatant fluid, of the same passage and 10 days after inoculation, had a titer of 10^{-3}. This was the same titer obtained by titrations of fluid from the first passage 4 and 8 days after inoculation. Other attempts at propagation of fibroma virus were unsuccessful. Line B virus from the original suspension and viruses from the third passage of lines A and B in cottontail testis cultures survived only a single passage in cultures of testes from domestic rabbits.

Negative results with other types of tissue. Virus multiplication did not occur in other types of tissue culture inoculated with original virus suspension of lines A and B. Both lines of virus were recoverable from the first but not from a second passage in human foreskin. Line B of fibroma virus was twice inoculated into cultures of monkey kidney cells. In both attempts the virus barely survived to a third passage, which represented only 3 tenfold dilutions of the starting suspensions.

Histology. A predominance of fibroblasts, surrounding islands of epithelial cells, characterized tissue cultures of both cottontail and domestic rabbit testes. Epithelial cells were more prevalent in the latter cultures. Fig. 2 illustrates proliferation of fibroblasts, as seen in an infected culture of cottontail testis. Uninoculated tissue cultures had a similar general appearance, for there was no evidence of cell destruction resulting from infection. Acidophilic, intracytoplasmic inclusions, irregular in size and shape and surrounded by a clear zone or halo, were abundant on first passage of virus in tissue cultures, whether of rabbit or of cottontail origin (Fig. 3). Occasionally, a single fibroblast might contain 2 inclusions. Inclusion bodies were less abundant on subsequent passage, but when present, they were concentrated in groups of closely adjacent cells. Similar cells in surrounding areas appeared normal. Fig. 4 represents a culture of fibroma virus (7th tissue culture passage) in cottontail testis tissue which had been inoculated 3 weeks previously. In this preparation inclusion bodies are associated with intracytoplasmic vacuoles. These vacuoles had a ground-glass appearance, apparently due to mucin-like contents. In common with the associated inclusions, they increased in prominence between the first (Fig. 2) and third weeks following inoculation. The vacuoles were encountered primarily in areas where the inclusions were also present and they were not observed in control, uninoculated cultures. Fig. 5 shows a section of a testis fibroma from an infected cottontail. It shows elongate vacuoles, some filled with a similar mucin-like substance, as well as 2 acidophilic, intracytoplasmic inclusions. Clear vacuoles, often in clusters and nearly filling the cytoplasm of certain cells, were occasionally found in both normal and infected tissue cultures. They were readily distinguishable from the vacuoles having a ground glass appearance and illustrated in Fig. 4. Using the periodic acid-Schiff (PAS) method, Fisher(5,6) described intracytoplas-

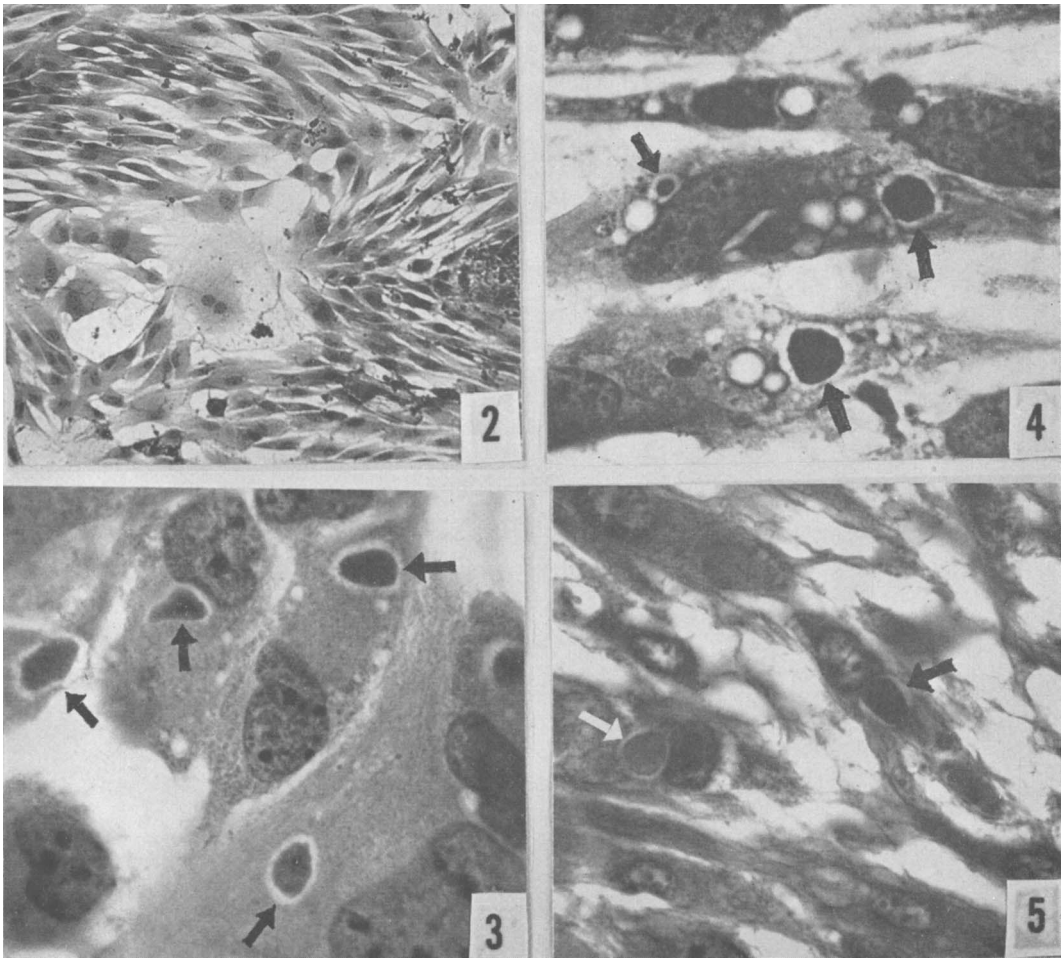

FIG. 2. Growth of fibroblasts from cottontail testis inoculated with 7th passage fibroma virus 5 days previously. (H & E 96×.)

FIG. 3. Acidophilic, intracytoplasmic inclusions (arrows) in tissue culture of cottontail testis, inoculated with fibroma virus 4 days previously. (H & E 1000×.)

FIG. 4. Intracytoplasmic inclusions (arrows) and associated vacuoles containing mucin-like substance. Seventh passage of fibroma virus, 3 wk after inoculation into tissue culture of cottontail testis. (H & E 1000×.)

FIG. 5. Section of fibroma which developed in testis of cottontail following inoculation. Inclusion bodies shown by arrows. (H & E 1000×.)

mic inclusions within fibroma cells of the corium which could not be stained with hematoxylin and eosin. He believed that these inclusions were possibly glycoprotein in nature and not related to the virus particles. Although PAS-positive inclusions were found in testis fibromas, they were not nearly as abundant as in fibroma cells of the corium in skin tumors of the same cottontail. So far I have had no definite evidence of the occurrence of this type of inclusion body in tissue culture preparations.

Discussion. Evidence is presented that fibroma virus multiplies in tissue cultures of cottontail testes. Two features of testicular fibromas, such as develop in cottontails following inoculation, are apparent in stained preparations of tissue cultures. They are acidophilic inclusions and secondly vacuoles filled with a mucin-like substance. The inclusions closely resemble those described by Feller, Enders, and Weller(7) in their study

of vaccinia virus in roller tube cultures of chick fibroblasts. This resemblance is possibly another expression of the relation of pox viruses to those of fibroma and myxoma(8). Propagation of fibroma virus in tissue culture offers a means of studying the nature of this tumor-inducing virus, its effect on living cells, and its transformation into strains of greater or lesser virulence.

Summary. (1) A mammalian tumor-inducing virus, that of the Shope fibroma, has been propagated in tissue culture. (2) Two lines of fibroma virus were each carried through 14 successive passages in tissue cultures of cottontail testes. (3) One line of virus was carried up to 4 passages in similar cultures of domestic rabbit testes. (4) Growth of virus was accompanied by formation of acidophilic, intracytoplasmic inclusions and by vacuoles containing a substance with a ground glass appearance.

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Received June 20, 1956. P.S.E.B.M., 1956, v92.

Effect of Diethylstilbestrol on Plasma 17-hydroxycorticosteroid Levels in Humans.* (22599)

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Evidence to indicate a stimulatory effect of estrogens on the pituitary-adrenocortical system in animals has been reviewed by Roberts and Szego(1). In the guinea pig, Zondek and Burstein(2) found increased urinary corticosteroid excretion following estrogen administration. On the other hand, Vogt(3) has measured directly adrenocortical hormones in the adrenal venous blood of the rat and rabbit, and found diminution of secretion following administration of hexoestrol in the rat but not in the rabbit. In the human, Gemzell(4) found elevated plasma 17-hydroxycorticosteroids during pregnancy, and suggested that such elevations are due to adrenal activation from the increased estrogen which occurs during pregnancy.

* Aided by grants from National Heart Institute (H-2376), and National Cancer Institute (CT-496) of National Institutes of Health, U. S. Public Health Service.

This study is concerned with plasma 17-hydroxycorticosteroid levels following diethylstilbestrol (stilboestrol) administration to human subjects, and was carried out because of the finding of markedly elevated levels in a patient with carcinoma of the prostate while on stilboestrol therapy.

Procedure. Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels(5), blood being drawn between 8:30 and 9:30 a. m. In this laboratory levels in 30 normal adults of both sexes were 14.4 $\pm$ 6.2 (mean $\pm$ S.D.) μg per 100 ml plasma (range 3.3 to 28.4); these values are similar to those of Bliss *et al.*(6).

Seven men with carcinoma of the prostate, one male (E.W.) with Addison's disease of 12 years duration, and one female (F.P.) who had undergone subtotal adrenalectomy for Cushing's syndrome were studied. In the patients with carcinoma of the prostate, pre-