VIROLOGY

Isolation of a Poxvirus from Stock Type 12 Adenovirus

Sweet and Hilleman[1] detected *SV*40 in seed stocks of adenoviruses, types 1–7, and Gerber *et al.*[2] reported the presence of viable *SV*40 in formalinized adenovirus vaccines. Yang and Melnick[3] described *SV*40 contamination of both adenovirus stock and vaccine. Such contaminations were attributed to the *SV*40 flora in many of the 'normal' monkey kidney cell cultures used for propagation of adenovirus. The report presented here describes the isolation of a poxvirus contaminant from a seed stock of adenovirus type 12. The poxvirus had been propagated by us in undetected form through many passages of the adenovirus in *HEp*-2 cells.

HEp-2 cell cultures, inoculated with stock adenovirus type 12, developed a cytopathogenic effect which was characterized by rounded and clumped cells and well-defined intranuclear inclusion bodies. Monkey kidney cell cultures, inoculated with the same undiluted adenovirus stock, developed a severe toxic reaction with marked cytopathogenic effect and intranuclear inclusion bodies; however, comparatively little infectious virus was produced. When the monkey kidney cell cultures were inoculated with the adenovirus diluted 1 : 10, plaques appeared in the cell sheet which resembled those of poxvirus. DNA-staining cytoplasmic inclusion bodies were observed by acridine orange fluorescence microscopy which were characteristic of those found in poxvirus-infected cells. In monkey kidney cell cultures inoculated with two-fold dilutions of the adenovirus stock (0·1 c.c.), the pox-like plaques appeared when the stock was diluted 1 : 4 or higher. The concentration of the poxvirus was $1{\cdot}6 \times 10^2$ plaque-forming units (P.F.U.) per ml. of adenovirus stock. The contaminating poxvirus (designated *TC*17) was detected in adenovirus stocks prepared on prior occasions from the same virus seed, as well as in the original vial of virus stock which was received from the custodian of the prototype virus. By diluting the adenovirus beyond its end-point in cultures of monkey kidney cells, the poxvirus contaminant was recovered free of adenovirus; and a pool of stock virus was prepared from this for further investigation.

When monolayers of *HEp*-2 cells were inoculated with *TC*17 virus, free of the adenovirus, plaques appeared and infectious virus was produced; however, the plaques appeared more slowly and were smaller and less easily detected than those produced in monolayers of monkey kidney cells. DNA-staining cytoplasmic inclusions were also observed in *HEp*-2 cells infected with *TC*17 virus and examined by acridine orange fluorescence microscopy.

Opaque white pocks were induced by *TC*17 on the chorioallantoic membrane of embryonated eggs. The surface of the pocks often appeared umbilicated. Three-week-old Swiss–Webster mice resisted intracerebral inoculation of 10^3 P.F.U. of *TC*17 virus; however, six of eight mice inoculated intracerebrally with 10^5 P.F.U. died 3 days after inoculation. Neither lesions nor symptoms were observed in mice inoculated intraperitoneally or intradermally with the virus. Rabbits developed a localized papule 2–3 days after intradermal inoculation of the poxvirus, and the lesions evolved through vesicle and pustule stages and were crusted over in 8–9 days.

While diethyl ether had no effect on the virus, it was inactivated by chloroform. Antiserum prepared against *TC*17 virus neutralized both vaccinia and monkeypox viruses, members of the same sub-group of poxviruses[4]. Rectangular or brick-shaped elementary bodies typical of poxviruses, were observed in the cytoplasm of infected cells by electron microscopy.

The biological and physical properties of the contaminating *TC*17 virus identify it with the poxvirus group; and, in view of its close serological relationship to vaccinia and monkeypox, it can be placed in the vaccinia-variola sub-group of poxviruses. This includes the viruses of monkeypox, ectromelia, cowpox, and contagious pustular dermatitis[4].

The discovery of poxvirus in the original stock of adenovirus type 12 and its inapparent but persistent propagations along with adenovirus in *HEp*-2 cells suggests the existence of an interference mechanism. Pereira[6] showed that adenovirus-infected HeLa cells produced a factor which inhibited multiplication of vaccinia virus in HeLa cells. Recent studies of Khoobyarian[7] have indicated that, under certain experimental conditions, the interaction of adenovirus with *RHF*-1 cells would result in cell resistance to vaccinia virus. It is conceivable that some similar mechanism, aided by the low concentration of the poxvirus, permitted it to exist undetected in the adenovirus stock. It is possible that a double infection resulting from the use of contaminated stocks could result in suppression of one, or its survival as an inapparent infection. The second virus would remain hidden until the conditions of propagation were changed in its favour. This may explain the emergence of poxvirus in monkey kidney cells.

The contaminating poxvirus may have originated from an occult infection in an earlier cell culture in which the adenovirus had been propagated or it may have resulted from a laboratory contamination prior to receipt in this laboratory.

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GENETICS

New Method of Isolating the Tetrads of Agarics

HITHERTO the usual technique for isolating tetrads from Agarics has been to detach a piece of gill tissue from a mature sporophore and then, with the aid of a micromanipulator, to pick off whole tetrads directly from the fresh gill. There are three main difficulties inherent in this method: (*a*) because of the high numerical density of tetrads on the gill, it is very difficult to be sure that no extraneous spores have been picked up by the micromanipulator needle until the spores are parked on the germination medium; (*b*) it is impossible to distinguish unripe from ripe tetrads, and since the former are removed from their basidia with great difficulty, many abortive attempts to pick up tetrads are frequently made before a tetrad is successfully removed; (*c*) in the case of *Coprinus*, autolysis of the gill can greatly limit the time available for isolation.

In the course of analysing tetrads from *Coprinus lagopus* (for the purpose of finding new centromere markers to be used for the extension of mapping) a new method has been developed. If a piece of gill tissue is taken from a mature sporophore, placed on a microscope slide and allowed to dry, the spores can be scattered on to the surrounding glass by sweeping the micromanipulator needle across the dried gill. Among the spores on the glass there is nearly always a high proportion of whole tetrads (Fig. 1), and these can very easily be removed from the