Abortive Infection of Hamster Cells by Polyoma Virus

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Mouse embryo cells are permissive for polyoma-virus replication. They support viral DNA replication and virus production and are finally killed.

In contrast, cells of the hamster cell line BHK 21/13* are non-permissive and undergo abortive transformation (Stoker, 1968). A small proportion of such cells are transformed and show permanently altered growth properties. Transformed cells contain at least part of the viral genome integrated into the cellular DNA (Westphal & Dulbecco, 1968; Sambrook, Westphal, Srinivasan & Dulbecco, 1968). Cellular DNA synthesis is probably necessary for the integration process (Todaro & Green, 1967).

Infection of stationary permissive cells, initially having a low rate of DNA synthesis, with polyoma virus results in a marked stimulation of cellular DNA synthesis (Dulbecco, Hartwell & Vogt, 1965). Studies on the interaction between polyoma virus and stationary BHK 21/13 cells are reported.

BHK 21/13 cells were maintained in a stationary state by using a method based on that of Fried & Pitts (1968) for mouse embryo cultures. Cells maintained in Eagle's medium containing 1% of calf serum for 5 days had low rates of DNA synthesis, low DNA polymerase activity and low thymidine kinase activity. DNA polymerase activity and thymidine activity increased 3–40-fold and DNA synthesis increased 200-fold when the cells were overlaid with fresh medium containing 10% of calf serum.

No significant increase in DNA synthesis, DNA polymerase activity or thymidine kinase activity occurred after infection of stationary BHK 21/13 cells with polyoma virus at a multiplicity of 50 plaque-forming units/cell. Infection of stationary mouse embryo cells under identical conditions resulted in 4–10-fold increases in DNA polymerase activity and thymidine kinase activity and 10–20-fold increases in DNA synthesis.

Viral-specific mRNA sequences were detected in polyoma-virus-infected BHK 21/13 cells by using a DNA-RNA competitive hybridization technique developed by Martin & Axelrod (1969).

Polyoma viral DNA replication could not be detected in BHK 21/13 cells (stationary or growing) or L929 cells, a mouse fibroblast cell line, after viral infection. However, limited viral DNA

replication did take place in polyoma-virus-infected hybrid BHK 21/13–L929 cells.

These results suggest that BHK 21/13 cells may lack a cellular function that is necessary for polyoma-virus replication.

G. A. N. is the holder of a Research Studentship from the Ministry of Education for Northern Ireland.

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Cause of the Heterogeneity of 'Silent' Plasma Cholinesterase Variants

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Rubinstein, Dietz, Hodges, Lubrano & Czebotar (1970) have reported that the homozygotes for the 'silent' cholinesterase (Ch_1^s) gene can be divided into two groups. One group (type 1 or S_1) has no cholinesterase except that originating from erythrocytes, and type 2 or S_2 has 2% of normal plasma cholinesterase activity. Thus type 1 and type 2 represent total absence and suppressed synthesis of plasma cholinesterase respectively.

The plasmas of two subjects homozygous for the apparent Ch_1^s gene were studied by a thiocholine assay procedure (Das & Liddell, 1970) with acetylthiocholine and butyrylthiocholine as substrates in the presence and in the absence of several inhibitors, by polyacrylamide-gel electrophoresis and by an immunoabsorption test. The results were compared with those obtained with normal plasma and a soluble preparation of normal erythrocyte cholinesterase (Das, Watts & Watts, 1971).

No activity could be demonstrated in either of the 'silent' samples with acetylcholine, butyrylcholine and benzocholine as substrates.

Both the samples had only about 1.5 and 0.8% of the activities in normal plasma with 5mM-acetylthiocholine and 7.5mM-butyrylthiocholine as substrate respectively. They hydrolyse acetylthiocholine slightly faster than butyrylthiocholine whereas the reverse is true for the normal plasma. But erythrocyte cholinesterase hydrolyses acetylthiocholine almost specifically.

Comparison of the Michaelis curves obtained with acetylthiocholine as substrate shows optimum

* Abbreviation: BHK cells, baby-hamster kidney cells.

substrate concentrations at 7.5, 7.0, 5 and 0.7–1.0mM for the sample 1 plasma, sample 2 plasma, normal plasma and erythrocyte cholinesterase respectively. With butyrylthiocholine as substrate, the values are 10, 7.5, and 7.5mM for the three plasma samples respectively. Values of $V_{\max}/2$ for the two 'silent' samples are similar to those for normal plasma, but differ from that for the erythrocyte cholinesterase by about five-fold when acetylthiocholine is the substrate.

With 5mM-acetylthiocholine as substrate the inhibitors 10nM-compound RO2-0683 [dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)trimethylammonium bromide], 0.3mM-sodium fluoride and 10mM-nialamide hydrochloride inhibit both the normal serum cholinesterase and the erythrocyte cholinesterase by 95–98% and 15–19%, by 80–90% and 12–18%, by 45–58% and 30–35% and by 34–40% and 19–25% respectively. On the other hand sample 1 plasma and sample 2 plasma are unaffected by these inhibitors. Compound C51 (1,5 bis[4 allyl-dimethylammonium]-pentan-3-one dibromide) at 5 μ M inhibits the acetylthiocholine-hydrolytic activities of sample 1 plasma, sample 2 plasma, normal plasma and erythrocyte cholinesterase by 28%, by 58%, by 14–20% and by 95–98% respectively. Similarly incubation with 6% butanol inhibits sample 2 plasma cholinesterase by 15% and erythrocyte cholinesterase by 20–30%, whereas sample 1 plasma cholinesterase and normal plasma cholinesterase remain unaffected.

Gel electrophoresis of the two 'silent' plasma on the 7.5% polyacrylamide discs at pH8.6 by the method of Ornstein (1964) and Davies (1964) shows five enzymically active zones when stained with acetylthiocholine with similar mobilities to those present in normal plasma. Erythrocyte cholinesterase preparations contain three bands with different mobilities from those in the plasma samples. A considerable amount of erythrocyte cholinesterase remains at the origin. The enzyme bands of the 'silent' samples show less activity against butyrylthiocholine, and the reverse is true with the normal plasma cholinesterase.

Sample 1 plasma possesses the immunological and electrophoretic properties of normal plasma cholinesterase. On the other hand sample 2 plasma differs from sample 1 plasma (i) in not neutralizing a specific antiserum and (ii) in being inhibited by 6% butanol and by 5 μ M-compound C51.

Although the inhibition value by compound C51 of sample 2 plasma may indicate the presence of an erythrocyte cholinesterase, other inhibition values and electrophoretic properties suggest that the 'silent' cholinesterase variants are mutants of plasma cholinesterase and provide further evidence that they are heterogeneous. Because of the complex properties of the 'silent' samples, the cause

of the heterogeneity must be treated with caution until better characterization of the purified enzyme is possible.

I am indebted to the Muscular Dystrophy Group of Great Britain, to the Endowment Fund of Guy's Hospital for financial support and to Dr D. C. Watts and Dr J. Liddell for their help.

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Cell Fusion without Viruses

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Aqueous dispersions of lysolecithin (lysophosphatidylcholine) can induce cell fusion and the formation of both homokaryons and heterokaryons (Poole, Howell & Lucy, 1970). Electron microscopy has now shown that fusion of avian erythrocytes by lysolecithin involves the establishment of small cytoplasmic bridges between cells. The polykaryocytes subsequently produced disintegrate rapidly owing to generalized membrane damage, but damage can be minimized by using lysolecithin in micro-droplets of glycerol trioleate. Only those regions of membrane in contact with the lipid droplets are then directly affected, and the droplets may be regarded as morphological equivalents of Sendai-virus particles. These experiments may be relevant to membrane fusion in the exocytosis of milk-fat droplets containing lysolecithin (Keenan, Morr, Olson, Yungmans & Patton, 1970).

The following procedure has been devised to determine the ability of defined chemicals to cause cell fusion. Hen erythrocytes in Eagle's medium or buffered saline at pH5.6 containing Dextran 60C (mol.wt. 77500) (80mg/ml) are treated with the test substances at 37°C for up to 2h, and bovine serum albumin is then added. Lysis accompanying cell fusion is thus minimized, except with aqueous lysolecithin, and the polykaryocytes remain intact for 24h. This allows the quantitative measurement of cell fusion by optical and electrical methods. Various degrees of cell fusion are seen with retinol, oleic, linoleic, linolenic, lauric acid glycerol monooleate, glycerol monolaurate and selachyl alcohol, and when Ca^{2+} ions are followed by F^{-} ions. Fusion apparently does not occur in this procedure with