SHORT ARTICLES

NON-PRODUCTION OF INTERFERING SUBSTANCES BY SERUM FROM PATIENTS WITH INFECTIOUS HEPATITIS

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INTERFERON and interfering substances with viral inhibitory properties are synthesised in cells in response to virus infection and have been found in sera and tissue extracts from laboratory animals and patients infected with a variety of viruses. Gledhill (1959) showed that serum obtained 3–4 days after intraperitoneal inoculation of mouse hepatitis virus (MHV) protects normal mice from the lethal effects of ectromelia virus. In another study it was found that both virulent and attenuated strains of MHV induced interferon production in normal and in partially hepatectomised animals (Mallucci, 1964–65). The lack of specific laboratory tests for human hepatitis virus prompted this attempt to demonstrate indirectly the presence of infectious virus by the induction of interfering substances in cultures of primary human embryo hepatocytes.

MATERIALS AND METHODS

The method used for the culture of primary human embryo hepatocytes is that described by Zuckerman, Tsiquaye and Fulton (1967), with the modification that the foetal calf serum used in the growth medium was inactivated at 56°C for 30 min. before use. Suspension cultures were prepared by trypsinisation of human embryo livers for 20 min. at room temperature; the cells were washed twice in the growth medium and resuspended in fresh medium. A seed of 8×10^5 cells per ml was placed in standard tissue culture tubes in 2-ml volumes and rolled at a speed of 6 r.p.h. at 35°C for 48 hr. A sample of human interferon was used as reference material. This had been prepared from white cell cultures and had a titre of 4000 when assayed by the inhibition of the cytopathic effect of HGP rhinovirus in human embryo lung cells.

Human embryo hepatocytes were tested for their ability to produce interferon-like substance by induction with Semliki Forest virus. The interfering substance produced, although in low titre, was capable of protecting the hepatocytes against the cytopathic effect of San Carlos virus 3 when 0.2 ml of a 1 in 32 dilution of the cell-free medium was added to the cell cultures 48 hr before the inoculation of 50 TCID₅₀ of the challenge virus.

The San Carlos virus 3 used as the challenge virus in these experiments was initially isolated by Davis (1961) from faecal specimens from young American Indian children admitted to hospital in Arizona with infectious hepatitis. Hatch and Siem (1966) found that the majority of the San Carlos isolates were neutralised by adenovirus type-3 antiserum, but that they differed from the prototype adenovirus in their homologous and heterologous neutralisation titres and by the haemagglutination-inhibition test. Zuckerman, Dunkley and Love (1968) observed also that the San Carlos viruses differed from prototype adenovirus 3 in the cytopathic changes produced in liver cell cultures and in their resistance to inactivation by heat.

Twenty-two acute and convalescent samples of serum were obtained from patients diagnosed clinically as suffering from virus hepatitis; 12 serum samples were from blood donors of the National Blood Transfusion Service known to have caused more than one case of hepatitis in recipients, and one was a freeze-dried sample of serum that had previously

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been used to produce hepatitis in human volunteers. Each serum in 0.2 ml amounts was inoculated into primary and suspension cultures of human embryo hepatocytes. After adsorption for 1 hr at 35°C the cells were washed once to remove residual serum. The foetal calf serum in the maintenance medium of these cultures was reduced to 5 per cent. The fluids from each culture were harvested at 2, 4, 18, 24 and 48 hr and 7 days after inoculation, centrifuged at 300*g* at 4°C for 10 min. to remove cell debris and stored at -55°C until required for testing. Test fluids in 0.2 ml volumes were added to 48-hr-old monolayers of primary human embryo hepatocytes and incubated at 35°C for 1 hr. Maintenance medium containing 2 per cent. foetal calf serum was then added, and the cultures were subsequently incubated for a further period of 24-48 hr. After the test fluid was removed, the cultures were washed twice with maintenance medium and challenged with 100 TCID₅₀ of San Carlos virus 3. The reference human interferon was included in parallel as a control. Cultures were stained with 1 in 1000 (w/v) solution of acridine orange in acetate veronal buffer, pH 4.2, without prior fixation, and were examined by fluorescence microscopy. In control preparations, at least 75 per cent. of the cells were affected by the virus.

RESULTS AND DISCUSSION

The human interferon reference preparation consistently protected the hepatocytes from the cytopathic effect normally produced by San Carlos virus 3. This observation is worth recording because of the absence of any published data on the susceptibility of adenoviruses to interferon (Ho, 1966). The San Carlos virus strain we used in this study was neutralised by adenovirus type-3 antiserum although differences from the prototype adenovirus strain are present. Adenovirus strains displaying antigenic variation and serological overlap have been described previously, but the significance of these antigenic differences within the adenovirus group is not understood (Parks *et al.*, 1967). No virus-inhibitory activity was demonstrated in any of the serum specimens we examined either in the acute phase or during the convalescent stage of the illness.

It is difficult to speculate on the reason for this uniformly negative result. One possibility is that, like naturally occurring rubella virus (Parkman *et al.*, 1966) and poliovirus (Isaacs, 1963), the virulent forms of the infectious hepatitis virus do not possess the ability to induce interferon production, whereas attenuated strains of rubella and poliovirus are capable of doing so. Another possibility is that our cell assay system may not have been sufficiently sensitive to detect the presence of very small amounts of interfering substances. The likelihood that our original clinical samples did not contain the infective agent is negated by the fact that at least some of the sera had caused serum hepatitis in more than one recipient of the serum. It may also be that the virus did not penetrate the hepatocytes. Finally, Wheelock (1967) in the United States examined multiple serum samples from 34 patients in all stages of acute and chronic hepatitis and also failed to find any evidence of virus-inhibitory activity.

SUMMARY

Twenty-two samples of serum obtained from patients suffering from infectious or serum hepatitis, 12 samples from blood donors known to have caused hepatitis in recipients and one sample of serum that had caused hepatitis in human volunteers were examined for their capacity to induce interfering substances, and thus, indirectly, for the presence of infectious virus.

No virus-inhibitory activity was detected with any of the sera examined.

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LONG-CHAIN MUTANTS OF *STREPTOCOCCUS FAECALIS*
 INDUCED BY ULTRAVIOLET IRRADIATION

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AMONG the streptococci, which are characterised as a genus by chain formation, the species *Streptococcus faecalis* is exceptional in forming short aggregates of 4-6 cells or no chains at all. The organism can be made to grow as a chain-forming phenotypic variant in the presence of a number of chemically unrelated compounds of which Suramin (Bayer 205) is the most useful (Lominski, Cameron and Wyllie, 1958). A strain was also found (NCTC 2400) which, although biochemically and serologically identical with *S. faecalis*, grew spontaneously in long chains. Both types of chain are readily broken down by filtrates of ordinary cultures and by lysozyme; lysozyme in turn was found to be powerfully inhibited by Suramin (Lominski and Gray, 1961).

It was assumed as a working hypothesis that separation of bacterial cells is an enzymic process; in the light of this, the enforced formation of chains by chemicals would be due to their inhibitory effect on the cell-separating enzyme, and strains that spontaneously formed long chains would represent enzyme-defective mutants. It seemed possible therefore that ultraviolet irradiation might produce chaining mutants from non-chaining strains.

Two recently isolated strains were used; in 18-hr broth cultures they appeared as single cells or aggregates of 2-4 cocci. Biochemically and serologically they were typical strains of *S. faecalis*.

An overnight fluid broth culture was spun at 2000*g*, washed and resuspended in saline to give a density of about 2×10^9 cells per ml; 0.5 ml of the suspension was placed in a sterile, disposable petri dish (Sterilin) and held for periods up to 60 min. at 24 in. (60 cm) distance from the radiation source (Hanovia 2537 AU, Model II, 12 in., 7 watt, U tube