

## TISSUE CULTURE OF ADULT LIVER BIOPSIES

SUSAN F. SULLMAN, A. J. ZUCKERMAN, PATRICIA F. TAYLOR, AND J. STUBBS (London School of Hygiene and Tropical Medicine and St. Mary Abbot's Hospital, London) The tissue culture method for human embryonic hepatocytes described by Zuckerman, Tsiquaye, and Fulton (1967) has been extended to fragments of human adult liver obtained from biopsies taken for diagnostic purposes.

The liver was cut into very small pieces and allowed to stand in 0.2% trypsin solution for 30 min. before stirring magnetically for five min. Small pieces of the softened tissue were washed and then teased out with two needles before implantation. Clotted mouse plasma was used as an adhesive. The tissue culture plates were incubated for about a week at 35°C in a dessicator containing 2% added CO<sub>2</sub>, and subsequently placed in Leighton tubes for examination at intervals by light and phase-contrast microscopy.

Two cell types could be readily distinguished after staining with acridine orange, namely, hepatocytes and fibroblast-like cells. The former were rounded with small nuclei and the cells were not infrequently binucleate. The cytoplasm appeared granular and foamy. The fibroblasts varied in shape and length and possessed an oval nucleus, and the cytoplasm stained a homogeneous dull orange in colour.

The cells grew out to form a confluent monolayer suitable for use after 10 to 14 days and the cytopathic effect of a number of viruses was studied, including adenovirus type 5, Sabin type 1 poliovirus, vaccinia cowpox, San Carlos viruses 3, 6, and 8, and infectious canine hepatitis virus.

The method described by Zuckerman, Kay, and Hockley (1967) for storing human embryonic liver cells in liquid nitrogen has been adapted for the adult tissue. The washed cells were suspended in growth medium containing 10% dimethylsulphoxide or 10% glycerol and 2 ml aliquots slowly frozen at -55°C for 1½ hr before being transferred to a liquid nitrogen container. The cells were recovered after storage for varying periods of time.

## REFERENCES

- Zuckerman, A. J., Tsiquaye, K. N., and Fulton, F. (1967). *Brit. J. exp. Path.*, **48**, 20.  
Zuckerman, A. J., Kay, H. E. M., and Hockley, A. B. (1968). *J. clin. Path.*, **21**, 109.

## LACK OF PRODUCTION OF INTERFERING SUBSTANCES IN INFECTIOUS HEPATITIS

PATRICIA E. TAYLOR AND A. J. ZUCKERMAN (Department of Bacteriology and Immunology, London School of Hygiene and Tropical Medicine) Interferon and interfering substances are synthesized in cells in response to many viral stimuli. These substances have been found in sera and tissue extracts from laboratory animals and patients infected with a variety of viruses. The uniform lack of specific laboratory tests for the human hepatitis virus, and the absence of definite evidence that this virus undergoes detectable replication in any laboratory system, prompted this attempt to demonstrate indirectly the presence of infectious hepatitis virus by the induction of interfering

substances in cultures of primary human embryo hepatocytes.

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 used in human volunteer experiments (supplied by Dr F. O. MacCallum) were examined. These were inoculated on to both primary monolayer (Zuckerman, Tsiquaye, and Fulton, 1967) and suspension cultures of human embryo hepatocytes, and adsorbed for one hour at 35°C. The cells were then washed once before the addition of maintenance medium. Tissue culture fluids, harvested at two, four, 18, 24, 48 hours, and seven days after inoculation, were added to fresh monolayers of primary hepatocytes. The test fluids and maintenance medium were removed 24 to 48 hours later. After washing twice with maintenance medium, the cultures were challenged with San Carlos virus 3, which is neutralized by adenovirus type 3 antiserum, although differences from the prototype adenovirus strain are present (Zuckerman, Dunkley, and Love, 1968). A reference human interferon preparation, supplied by Dr N. B. Finter, was used in parallel in every test. The test was read when approximately 75% of the cells in control virus preparations were affected.

The human interferon reference preparation consistently protected the hepatocytes from the cytopathic effect normally produced by San Carlos virus 3. This is of interest because of the absence of published data on the susceptibility of adenoviruses to interferon (Ho, 1966). However, none of the sera examined induced the production of detectable amounts of interfering substances under the present experimental conditions. Wheelock, Schenker, and Combes (1968) detected no virus-inhibiting activity in multiple serum samples from 34 patients in all stages of acute and chronic hepatitis. Both these studies establish an important negative finding in the problem of human infectious hepatitis, and indicate that, by current techniques, the induction of interferon or interfering substances does not provide a diagnostic tool for this disease.

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## REFERENCES

- Ho, M. (1966). In *Interferons*. Ed. Finter, N.B. North Holland Publishing Co., Amsterdam, p. 24.  
Wheelock, E. F., Schenker, S., and Combes, B. (1968). *Proc. Soc. exp. Biol. Med.*, **128**, 251.  
Zuckerman, A. J., Dunkley, L. J., and Love, G. J. (1968). *Brit. J. exp. Path.*, **48**, 235.  
Zuckerman, A. J., Tsiquaye, K. N., and Fulton, F. (1967). *Brit. J. exp. Path.*, **48**, 20.

## PRECISION OF URINE:WHOLE BLOOD AND URINE:PLASMA RATIO FOR ALCOHOL

G. MUIR AND M. DAY (Luton) A comparison of these ratios was made. The urine: blood mean was 1.23, standard deviation 0.19. The figures for urine: plasma were mean 1.09, standard deviation 0.09.

Evidence was presented that the use of plasma offered no improvement over urine: blood ratios in determining plasma alcohol concentration.