HEPATITIS B

Antigen Subtypes

from our Medical Virology Correspondent

INCOMPLETE fusion of precipitin lines and the formation of spurs when the same sera containing hepatitis B antigen are tested against different antisera indicate that the two adjacent antigens are related but not identical. Yet when the same antigens are tested against other antisera no spurs are obtained and the precipitin lines give lines of immunological identity (G. L. Le Bouvier, *Amer. J. Dis. Child.*, **123**, 420; 1972). Hepatitis B antigen thus does not represent a single entity.

The group specific antigen, termed *a*, is common to all hepatitis B antigen-positive sera and there are at least two principal antigenic sub-specificities, *d* and *y*, which behave in a mutually exclusive manner but are carried on the same antigen particles bearing *a*. The same antigenic subdeterminants persist during the course of antigenaemia in patients with hepatitis B as well as in persistent carriers of the antigen. These subdeterminants seem to be dictated by the infecting agent and they have been found to breed true in experimental transmission to human volunteers (G. L. Le Bouvier *et al.*, *J. Amer. Med. Ass.*, **222**, 928; 1972). This is further confirmed by the observation of only one subtype of antigen in localized outbreaks of hepatitis B infection studied in appropriate epidemiological settings (J. W. Mosley *et al.*, *Amer. J. Epidem.*, **95**, 529; 1972).

The discovery of the *ad* and *ay* subtypes does not, of course, preclude the possibility that there may exist, within each subtype, other inherited differences that are dependent on the *d-y* dichotomy, such as *w* and *r* which are associated with either *d* or *y*, or others that are independent of *d* and *y*.

An interesting geographical distribution of hepatitis B antigen subtypes is now emerging. W. H. Bancroft and his colleagues from the Walter Reed Army Institute of Research (*J. Immunol.*, **109**, 842 ; 1972) have detected two additional antigenic determinants named *w* and *r* (*W*alter *R*eed); subdeterminant *w* (*adw*) is prevalent in North America and in Western Europe, whereas *r* (*adr*) is much commoner in the Far East, South-east Asia and the Middle East. The subdeterminants *ay* (S. Saidi *et al.*, *Lancet*, ii, 1377; 1972) and *ayw* are peculiarly common among healthy carriers and patients with hepatitis B infection in the Middle East and Eastern Mediterranean and constitute an unusual zone of *y* predominance to the virtual exclusion of subtype *d*.

What has happened to the missing subdeterminant *ayr*? P. V. Holland and his colleagues (*Hepatitis Scientific Memoranda*, November 1972) have described hepatitis B antigen with the "alleles" *ayr*, and this subtype also seems to be much more prevalent in the Far East or among patients who had visited Asia. The geographical distribution of the subtypes in Eastern Europe, Africa and South America is not known at present.

The relationship of the subtypes of hepatitis B antigen to different clinical expressions of infection is being investigated. Preliminary results suggest an uneven distribution between acute and chronic forms of infection with hepatitis B virus and indicate a greater prevalence of subtype *ad* among asymptomatic carriers of the antigen in Western Europe and North America and a marked preponderance of *ay* in acute hepatitis among drug addicts and patients on maintenance haemodialysis in these geographical areas.

Some of the important questions which remain to be answered include the extent of cross-immunity between the two principal subtypes, their relative pathogenicity and persistence and their geographical distribution throughout the world.

VIRUSES

Inhibition and Promotion

from our Cell Biology Correspondent

PERMISSIVE cells lytically infected by SV40 and non-permissive cells transformed by this virus offer a considerable advantage to anybody investigating transcription in eukaryotes, for the pattern of synthesis of viral RNAs in such cells can be monitored by hybridizing RNA from the infected cells with SV40 DNA which can be obtained pure. Ossowski and Reich (*Virology*, **50**, 631 ; 1972) made use of this system to investigate the way in which two nucleoside analogues, 5-fluorouridine (FUrd) and 5-bromotubercidin (BrTu), inhibit transcription. FUrd irreversibly inhibits RNA synthesis in BSC-1 monkey cells, but when these cells are exposed briefly to FUrd before infection by SV40, viral DNA is synthesized and T antigen is induced. Likewise FUrd does not, as judged by hybridization experiments, alter the pattern of transcription of SV40 RNA in 3T3 cells transformed by SV40. By contrast BrTu inhibits synthesis of SV40 RNA in SV/3T3 cells. As Ossowski and Reich point out, these data are consonant with previously reported experiments which indicate that FUrd does not block messenger RNA synthesis even though it does block ribosomal RNA synthesis, whereas BrTu blocks both messenger and ribosomal RNA synthesis.

Groups investigating the biology of polyoma virus, a close relative of SV40, seem to grow accustomed to the frustration of never, or seldom, having a sufficient supply of concentrated stocks of the viral particles to meet their various needs. And, for example, the failure to obtain easily large amounts of this virus is at least in part responsible for one's ignorance of the chemistry of the structural proteins of the polyoma virus particle. Obviously any suggestion as to how to increase the yield of polyoma virus from infected cultures merits attention. According to Thorne (*J. Gen. Virol.*, **18**, 153 and 163 ; 1973) the yield of polyoma virus from infected cultures of Balb/3T3 cells depends on the stage in the cell cycle that the cells have reached when they are infected. Maximum yields are obtained when the cells are infected at, or near, the beginning of the G1 phase.

Thorne finds that the yield per cell of polyoma virus decreases as the density of the Balb/3T3 cell culture increases even though close cell to cell contact *per se* does not reduce the yield of virus. After exposing confluent cultures in which most cells are not dividing rapidly to trypsin and replating the cells, both the adsorption of infecting viral particles and the yield of progeny particles increase. This and other experiments, including the addition of fresh serum to confluent cultures at various times before infection, led Thorne to suggest, first, that dividing cells offer the most favourable environment for the replication of polyoma virus and, second, that a trypsin-sensitive component may accumulate at the cell surface of resting cells and reduce the capacity of polyoma virus particles to adsorb to the cells. Thorne also noticed that the extent of cell DNA synthesis in cultures of clones of Balb/3T3 cells infected by polyoma virus either did not change or was inhibited. Whether this pattern results from induction of cell DNA synthesis in those cells productively infected and a compensatory inhibition of cell DNA synthesis in those cells non-productively infected remains to be seen. But it is clear that the generalization that infection by polyoma virus universally induces cell DNA synthesis has to be taken with a pinch of salt.

Because these experiments suggested that polyoma virus replicates efficiently only in dividing Balb/3T3 cells Thorne set up synchronized cultures using three methods, mitotic cell harvest, thymidine blockade and colcemid blockade, to establish synchrony. He then infected the cells at various stages in the cell cycle with polyoma virus. In all cases he found the maximum yield is obtained from cells that are infected in the G1 stage of the cell cycle although the period of maximum susceptibility cannot, from the data obtained, be more precisely defined.