European tumour virology at Aviemore

from Elizabeth Rogers

THE momentum of tumour virus research is such that pauses for breath are essential if one is not to be left gasping behind. So it was that an informal meeting of European tumour virologists at Aviemore, Inverness-shire from April 3–5 provided an opportunity for gathering together the threads of recent progress. The meeting was organised by Professor J. Subak-Sharpe (Institute of Virology, Glasgow) and the sixty or so short contributions covered research into all major groups of tumour viruses. Outstanding were the descriptions of the use of restriction enzymes as tools for analysing the genomes of DNA viruses, and the use of pieces of DNA as infective agents in order to define, for instance, the size of the transforming 'gene'.

The small DNA papova viruses have, not surprisingly, yielded most rapidly to analysis with restriction endonucleases. Linear SV40 DNA produced by *Escherichia coli* R₁ endonuclease can transform rat embryo kidney cells (P. J. Abrahams, State University, Leiden), although it can no longer cause productive infection in BSC-1 monkey kidney cells. Transforming activity of restriction fragments smaller than the whole genome is being investigated. A new SV40-specific antigen reported by C. de Vaux St Cyr (Institute of Cancer Research Villejuif) will provide food for thought for genome analysers, already overburdened with gene products to fit into too little virus DNA. This antigen—named 'C'—is cytoplasmic and distinct from T antigen in several other respects. Those who think that polyoma might behave like SV40 will be increasingly disillusioned by reports like that of R. Kamen (Imperial Cancer Research Fund, London), who has found that late in polyoma infection, the major virus-specific RNA is a total transcript of the minus strand—unlike the situation in SV40-infected cells. A transcription map of the polyoma genome is now available, derived from analysis of eight fragments formed by *Haemophilus parainfluenzae* endonuclease II (HpaII). The origin of replication has been localised in fragment 5 by M. Fried and L. Crawford (ICRF, London). There is a small region of homology with SV40 DNA.

Larger DNA viruses are more complex substrates for restriction analysis, but already considerable progress with adenoviruses (Ad) 2 and 5 has been made. Using a combination of EcoRI and HpaI restriction enzymes, P. H. Gallimore (Medical School, Birmingham) has analysed the parts of the adenovirus genome which are present

in Ad2-transformed cells. In some lines as little as 14% of the genome remains. J. F. Williams (Institute of Virology, Glasgow) also reported that two Ad5-transformed cell lines have, respectively, 2½ and 5½ copies of the left end, representing about 30% and 40% of the genome. These findings relate to the question of how much adenovirus DNA is essential for transformation. F. L. Graham (State University, Leiden) has treated rat embryo cells with sheared adeno-DNA. Ad5-DNA will transform these cells even when only about 1×10^6 daltons in size. If half-molecules of Ad5-DNA are made, the transforming activity can be localised in the GC-rich half, and after restriction with EcoRI, it is further localised in the large A fragment, which includes the left end of the molecule. Considering that transformed cell lines exist which only contain the terminal 12% of the left half of the genome, transforming activity must be confined to this site.

Although the problems presented by herpes and Epstein-Barr viruses seem much more immense, these viruses are slowly yielding to the same methods as have been used successfully with small DNA viruses, and their role in human carcinomas is becoming clearer at the same time. Herpes virus (HSV) is a potential candidate for a human tumour-inducing virus. Patients with different carcinomas have been shown to carry HSV-specific non-virion antigens in their sera, and many studies of the transforming potential of HSV or HSV-DNA are now in progress. For example, infection of human embryonic lung cells with HSV is abortive in certain conditions, and cells can become stably transformed (K. Munk, Institute of Virology, Heidelberg). HSV cannot be rescued by cocultivation, so that this may be a good experimental system for studying latency of HSV in human tissue. Another system has been developed by J. C. M. Macnab and N. M. Wilkie (Institute of Virology, Glasgow), who have obtained focal transformation of rat embryo cells by HSV types 1 and 2, as well as by temperature-sensitive (*ts*) mutants and sheared DNA derived either from wild type or from *ts* mutant viruses. As with adenovirus and SV40 DNAs, infectivity of DNA is far more sensitive to shearing than is transforming activity. Sheared HSV-DNA of 5.7×10^6 daltons (whole genome is 100×10^6 daltons) will induce transformation. J. K. McDougall (Medical School, Birmingham) demonstrated by *in situ* hybridisation that among a population of human cervical carcinoma cells, only very few contain detectable amounts of HSV-DNA, which may explain why whole virus cannot be isolated from the carcinoma. *In situ* hybridisation, be-

cause it allows detection of viral genomes at a cellular level, provides another dimension in the overall analysis of the biochemistry of tumours.

H. Diggelman (Swiss Institute of Cancer Research, Lausanne), introducing a series of papers on RNA viruses, said that a central problem in understanding the function of RNA tumour viruses is still that of determining whether the 70S RNA genome is polyploid. 70S RNA is not found attached to polysomes, nor even in the first budding virus particles, but is apparently assembled later. J. T. Parsons (University of Zurich) has evidence from fingerprint analysis that the genome size is about 3×10^6 daltons—the size of one subunit. Conclusive proof that avian leukaemic myeloblasts contain at least one complete integrated avian myeloblastosis virus (AMV) genome was provided by H. Ogura (Robert Koch Institute, Berlin) and F. Lacour (Institute Gustave Roussy, Villejuif) who have found that AMV production can be induced after infection of helper factor-negative chick cells with leukaemic myeloblast DNA. Similar results were got with wild type or *ts* mutant Rous sarcoma (RSV)-infected cell DNA. The minimum infective size suggests that the information from one RSV subunit may be sufficient. Viral mutants are necessary additional tools in these experiments. R. R. Friis (Robert Koch Institute, Berlin) has isolated representative mutants of the whole replicative cycle in RSV, including one which does not assemble reverse transcriptase into the virion.

Another aspect of RSV transformation was discussed by A. Vaheri (University of Helsinki), who has found that a type-specific glycoprotein, which is normally an integral part of the chick fibroblast cell membrane, disappears on transformation of the cells by RSV. If a *ts* virus mutant is used, the glycoprotein reappears at the non-permissive temperature. W. F. H. Jarrett (University of Glasgow) has studied the horizontal transmission of feline leukaemia virus (FeLV), and the incidence of leukaemia itself as compared with FeLV antibodies in different cat populations. He concludes that one of the primary events in leukaemia may be immunodepression of the response to FeLV antigens themselves. Significantly, the existence of horizontal transmission may allow the development of anti-FeLV vaccines (see *Nature*, **248**, 230; 1974).

Certainly this meeting served to demonstrate again the increasing finesse of the techniques being used to study tumour viruses. One hopes that probing into the mechanism of transformation in these ways will lead to an understanding of the spontaneous events which produce tumours.