and minus strand; these can then be separated by polyacrylamide/gel electrophoresis and studied separately.

First, the plus strand was completely degraded with both ribonucleases $T_1$ and A and the two sets of oligonucleotides were sequenced. This was helped by the fact that the 2′ or 3′ terminal phosphate is derived from the $\alpha$ phosphate of the ribosetriphosphate incorporated in the nearest 3′ neighbour position. Because it was possible to grow molecules in a medium with just one of the triphosphates labelled in this position with $^{32}P$, it was easy to detect the residues in the oligonucleotides with this nucleotide next to them in the 3′ position. In this way the nearest 3′ neighbours of all bases can be determined. This in itself gave most of the information necessary to sequence the oligonucleotide fragments, and a complete catalogue of the sequenced $T_1$ and A digest fragments from the plus strand was thus obtained. The method was then applied to the 1:1 complex of both strands and a combined catalogue for both was found. It was then a simple matter to subtract the plus strand catalogue from the combined catalogue to obtain that of the minus strand.

The nearest neighbour information together with partial overlaps between products obtained from the two sorts of enzyme and the fact that the plus and minus strand fragments were complementary enabled the fragments to be assembled into sixty-six extended sequence blocks, and the problem remained to place these blocks in the correct order. The nearest neighbour information severely restricted the possibilities but did not point to a unique solution. To eliminate ambiguities it was necessary to obtain information concerning the order in which the blocks occurred along the chain. This was accomplished by exploiting the ability of polyacrylamide gels to resolve RNA according to molecular weight. The replication system was set up to produce only minus strands and aliquots were removed and replication quenched at different times after initiation. This resulted in a set of partially completed molecules each of which started at the 5′ end. These were pooled and resolved on polyacrylamide gels. The resulting distribution was divided into twenty-two fractions and each was degraded by ribonuclease and fingerprinted. It was thus possible to test each fraction for the presence or absence of relevant enzymatic digestion products. If an oligonucleotide was absent it was clear that it must occur further down the chain than the point at which the RNA in that particular fraction was terminated. In this way it was possible to determine the order of the extended sequence blocks along the chain and to assemble them into a unique sequence.

The payoff of these elegant experiments comes from a close inspection of the final sequence. This was found to contain several complementary runs of nucleotides capable of forming helical regions. These helical regions tended to occur in positions along the chain which are resistant to enzymatic digestion. The authors point out that if one strand has such potential structure so must the other and that the two strands are, in a sense, mirror images. They also observe that if certain nucleotide sequences are favoured by the selection pressures which produced MDV-1, there would be a tendency for them to exist in both strands. Once this occurs there must necessarily be complementary regions in both chains leading to the possibility of secondary structure. Thus secondary structure in such molecules must be seen as a natural consequence of selection for molecules

# Immunochemistry of Hepatitis B Antigen

THE discovery of hepatitis B antigen has provided a valuable laboratory test for potential infectivity of blood and other material, but, in spite of considerable research, the biological nature of the antigen remains unknown. Morphological studies of the antigen by electron microscopy after negative staining revealed a plethora of structures including small spherical 20 nm particles. Some of these particles have several features in common with negatively stained virus particles and the finding of about 5% RNA in partially purified antigen (Jozwiak and colleagues, *Nature new Biol.*, **229**, 92; 1971) suggested a close affinity between the antigen and animal RNA viruses. The failure to confirm so far the presence of RNA in hepatitis B antigen, however, has led to several alternative proposals on the nature of the antigen.

The view that the antigen is composed of normal host-coded material such as serum proteins, assembled through the stimulus provided by infection with hepatitis B virus, and the background of genetic factors has led Blumberg and his colleagues (*J. exp. Med.*, **134**, 3205; 1971) to suggest that the antigen is a unique infectious agent which has the characteristics of an inherited serum protein polymorphism (*Nature new Biol.*, **234**, 226; 1971). Another view is that the antigen is a specific protein or lipoprotein produced by liver cells infected with hepatitis B virus. But the most likely proposition is that the antigen represents excess virus coat material, partly in the form of aggregates of protein subunits, and that the inner core of the 42 nm particle may be the infectious agent.

Biochemical dissection of the antigen now seems to provide further clues. Burrell and his associates report in *Nature New Biology* next Wednesday (June 27) the finding of significant amounts of carbohydrate in the 20 nm antigen particles. They consider it possible that either the carbohydrate is necessary for maintaining the structure and functional integrity of the antigenic determinants or that the carbohydrate itself constitutes an important antigenic determinant. This raises two intriguing possibilities. First, the carbohydrate might have a novel haptenic specificity which is either virus-coded or virus-induced host cell-coded, or, second, the carbohydrate (and indeed some lipoprotein components) might simply be derived from the host cell membranes as the mature virus particles are released by budding.

The incorporation of host specific antigenic material in the envelope of the virus does not alter the specificity of the viral antigens nor the biological properties of the virions. There may, however, be some antigenic similarities between the virus carbohydrate hapten and the carbohydrate antigens of normal cell surfaces, or between the virus protein and the protein moiety derived from the host, leading to the establishment of partial tolerance because of the antigenic similarity between hepatitis B antigen and "self" antigens. Tolerance may play a part in establishing the persistent carrier state. Another possibility is that antigen specificities which consist partly of host-derived material and partly of virus-derived material may elicit antibodies against such antigen complexes which in turn will react with the host cells, especially with virus infected cells. Such a mechanism may operate in the immunopathogenesis of liver damage in acute viral hepatitis (Farrow *et al.*, *Br. med. J.*, **2**, 693; 1970) and in chronic liver disease associated with hepatitis B infection (Zuckerman, *Immunopathology*, **6**, Schwabe, 1970, p. 436).