lack about ten bi-armed chromosomes derived from the A9 parent. It will be bad luck if future work does not pinpoint the crucial chromosomes.

These results with hybrids fly in the face of earlier reports that the fusion of a cancer and a non-cancer cell gives a malignant hybrid. Does this mean that the A9 cells possess some special property which suppresses malignancy and which is not shared by other non-malignant cells? One way to find out, of course, is to repeat the experiments with other non-malignant cell types. But what if it turns out that A9 is unique in this respect? A9 is a cell line that has been selected over several generations for the ability to continue to metabolize and grow in the presence of 8 azaguanine, an analogue of the base guanine which usually stops cell growth by inhibiting the synthesis of nucleic acid. It may be that the ability of A9 to suppress cancer is correlated with its ability to metabolize in the presence of 8 azaguanine. If so, this would open up a direct approach to the biochemical mechanisms involved in the suppression of malignancy.

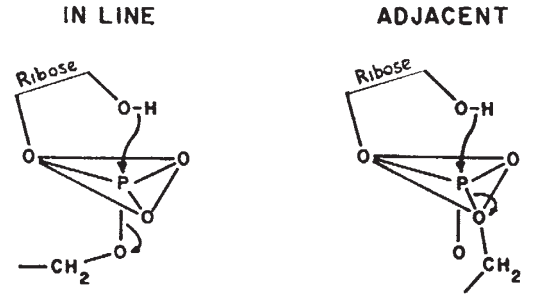
The experiments with the hybrids of A9 and Ehrlich ascites cells also prove that the expression of histocompatibility antigens can also be suppressed. In the A9-SEWA and A9-MSWBS hybrids, both parental histocompatibility antigens are fully expressed. This is the classical case of co-dominance. In each type of hybrid, the A9 cell contributes the H-2^k antigen and the SEWA and MSWBS cells the H-2^s antigen. Among other things, this explains why Harris and his colleagues had to test the hybrids for malignancy in mice heterozygous for these two antigens. The A9 and Ehrlich ascites hybrids, however, turned out to have weak histocompatibility antigens. The H-2^k antigens of the A9 parent had somehow been largely suppressed by the Ehrlich ascites parent, which on its own shows very weak antigenicity. The most likely explanation is that during its eighty years of laboratory manipulation, the Ehrlich cell has evolved a mechanism for suppressing its own histocompatibility antigens and, of course, in a hybrid containing the Ehrlich cell, this same mechanism would suppress the expression of the antigens of the other partner. The Ehrlich ascites cell might, for example, produce a substance which simply smothers the antigens on its surface. What makes this explanation particularly appealing is the discovery that in the Ehrlich ascites–A9 hybrids not only are the H-2^k antigens of the A9 cell suppressed, but so are the FMR antigens which are present probably because the A9 cell carries a leukaemia virus.

ENZYMES

The Five-fold Way

from our Enzymology Correspondent

THE difficulties in interpreting enzyme mechanism are nowhere better shown than by the example of ribonuclease. This small and tractable enzyme has received the full attention of X-ray crystallography and conventional chemistry, yet still nobody knows



how it works. There are various theories, but the evidence necessary to choose between them is elusive.

Meanwhile, background knowledge of nucleotide chemistry is growing quickly, and it is now possible at least to frame the chemical requirements for any ribonuclease mechanism more precisely than was possible a few years ago. In a recent paper, David Usher of Cornell University has shown that all the suggested mechanisms for the enzyme can be classified according to the geometry of the P—O bond breaking and making which they invoke (*Proc. US Nat. Acad. Sci.*, **62**, 661; 1969). There are only two classes, an "in-line" displacement and an "adjacent" displacement (shown in the diagram). This distinction has rarely been made explicit in the past, but most of the published mechanisms nevertheless carry an implicit geometry: for example, a mechanism is of the adjacent type if it uses the same group as a general base for the 2′-OH and then as a general acid for the departing 5′-OCH_2.

Work by Westheimer's group has led to a set of preference rules for the reactions of phosphorus esters, and among them are the following: (1) Hydrolysis involves a pentacoordinate species with the geometry of a trigonal bipyramid (three basal positions and two apical). (2) A pseudorotation can convert apical positions into basal, and two of the basal into apical. (3) Groups enter and leave via apical positions only. (4) A five-membered ring (for example, the intermediate cyclic phosphate in ribonuclease action) spans one basal and one apical position.

With these rules, it can be said that an adjacent mechanism cannot be concerted, for the attacking 2′-OH will necessarily approach the P atom at an apical position, so the basal 5′-O atom cannot depart until a pseudorotation has made it apical. An in-line mechanism could be concerted, on the other hand, though it would probably require simultaneous general base catalysis towards the 2′-OH, and general acid catalysis towards the 5′-O, presumably supplied by ribonuclease's two active site imidazole groups.

Given that an adjacent mechanism involves pseudorotation of a pentacoordinate intermediate, it is worth asking whether the process could happen fast enough to satisfy the observed rate of reaction. In a preliminary calculation, Hammes has recently shown that if the ratio of the pK_a of the 2′-OH to that of the enzyme base were 10^{-7} (as is probably the case) then the formation and rotation of the pentacoordinate intermediate may need to occur at rate constants as high as 10^{10} or 10^{11} s^{-1}. This is indeed very fast, and David Usher is currently trying to force the issue by designing a substrate with hindered pseudorotation, in the hope that a study of its hydrolysis by ribonuclease will provide an experimental distinction between the "in-line" and "adjacent" mechanisms.