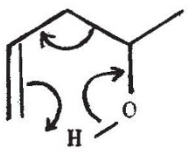
REACTION MECHANISMS

Where is the Proton?

from our Biological Chemistry Correspondent

KINETIC isotopic effects provide a specialized tool for investigating transition states of reactions which involve slow proton transfers. At the qualitative level, the technique of exchanging hydrogen for deuterium has many successes to its credit, not least in the field of enzymatic mechanisms. The problems emerge when attention is directed to the significance, if any, of the quantitative aspects of primary isotopic effects.

The early theoretical appraisals, notably due to Westheimer and to Biegeleisen, developed the concept that the magnitude of the kinetic change observed on isotopic substitution should reflect the extent of hydrogen transfer in the transition state: "maximum" effects correspond to those reactions in which the hydrogen is symmetrically located between the donor and acceptor groups in the transition state. Although this idea has been widely applied, direct supporting data have been sparse and the concept has been challenged often, most recently by F. G. Bordwell and W. J. Boyle (*J. Amer. Chem. Soc.*, **93**, 512; 1971) who searched in vain for a simple correlation between isotopic effect and extent of proton transfer.



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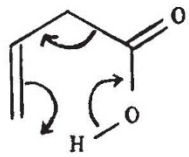

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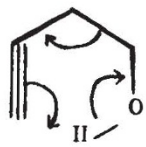

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H. C. Kwart and M. C. Latimore (*ibid.*, **93**, 3770; 1971) have now provided strong validation of Westheimer's concept by investigating three gas-phase reactions. The thermolytic fragmentation of 4-penten-1-ol (1), 3-butenoic acid (2), and 3-butyn-1-ol (3) all proceed through concerted bond-reorganization processes which unquestionably involve transfer of hydrogen from oxygen to carbon—formally indicated by the "curly" arrows, but probably sigmatropic processes. In spite of this similarity, the three cases provide a significant variation both of the acidity of the hydrogen being transferred and in the electronic structure around the acceptor-carbon. If therefore these factors influence the isotopic effect, differences should become apparent.

The rates of all three reactions, measured over a range of temperatures, show a 2-fold to 2.5-fold rate retardation on replacement of hydrogen by deuterium. "Maximum" values for the isotopic effects were calculated on the assumption that the zero-point energy difference, O-H *vs* O-D, alone determines the kinetic isotope effect. In each case, observation and calculation agree within the limits of experimental error.

These results thus provide one of the strongest supports for the continued use of kinetic deuterium isotopic effects as a criterion for concertedness in hydrogen transfer reactions.

HEPATITIS

Active Immunization

from our Medical Virology Correspondent

IT has been repeatedly demonstrated in many parts of the world that the injection of normal human immunoglobulin (gamma-globulin) during the incubation period of infectious (epidemic) hepatitis can prevent jaundice among contacts. Although the incidence of the icteric form of hepatitis was thus reduced, infection was not prevented—it was attenuated to a variety of hepatitis without jaundice or to the subclinical form of the disease and faecal excretion of the virus still occurred.

The precise mode of action of immunoglobulin is not completely known but it is presumed that partial passive, and therefore short term, immunity against infectious hepatitis is obtained by the antibodies present in the globulin prepared from large pools of plasma. Active immunity may, of course, be acquired through subclinical infection, so-called passive–active immunity.

The problem with serum hepatitis, on the other hand, seems to be entirely different, and immunoglobulin has not reduced significantly the incidence of post-transfusion hepatitis. The discovery of the association between Australia antigen and serum hepatitis has provided the crucial key for studies of this form of hepatitis. S. Krugman and his associates (*J. Amer. Med. Ass.*, **217**, 41; 1971) now report the results of limited studies in volunteer children on active immunization against serum hepatitis caused by the MS-2 strain. Thirty-nine children were divided into two groups: one group of twenty-five susceptible children were injected with MS-2 serum; the second group included fourteen susceptible children who received either one or two inoculations of heat inactivated preparation of a 1 in 10 dilution of MS-2 serum followed by an inoculation of untreated infective serum 4 to 8 months later. It was found that hepatitis induced by MS-2 serum was prevented by active immunization and that two spaced inoculations were more effective than one. Nevertheless, one inoculation gave enough protection to prevent some cases of serum hepatitis and to modify others. Krugman and his colleagues emphasize that these obser-

"Unblocking" Sera in Anti-tumour Immunity

DURING the past few years evidence has accumulated that animals and human patients with progressively growing tumours often have immune cells that are capable of reacting against their own tumour cells, as shown by cytotoxicity tests *in vitro*, but they also have in their sera factors that can specifically block the anti-tumour reactions. To increase anti-tumour immunity it would be useful if ways could be found for overcoming such blocking effects. The possibility that another group of serum factors can "unblock" immune reactions against tumours arises from the work of the Hellströms and their associates on sera from mice with regressing Moloney sarcomas, and of Bansal and Sjögren on polyoma virus-induced tumours in inbred rats. The latest report from these last authors will appear in next Wednesday's *Nature New Biology*.

Bansal and Sjögren have found that when sera from rats immune to polyoma tumours are mixed with homologous blocking serum, they counteract the inhibition produced by the blocking serum on lymphocyte-mediated cytotoxicity against polyoma tumour cells *in vitro*. Still more remarkable, such sera inoculated into five rats bearing transplanted polyoma tumours brought about regression of the tumours in four of the animals. These effects are not caused by anti-viral antibody, and it seems unlikely that they are caused by serum-mediated autotoxicity because the transplanted cells grew into palpable tumours before regressing—such regression is never seen in untreated rats. Further work is required to establish how common unblocking activity in serum is, whether it is caused by a particular kind of antibody, and how it unblocks. One interesting possibility is that blocking is caused by antigen–antibody complexes, with which unblocking could react. Whatever the mechanism, there is no doubt that unblocking is of general interest as well as potential therapeutic significance.