# SESSION VII

# III. INVESTIGATIONS OF ISOTOPE EFFECTS IN BIOCHEMICAL SYSTEMS

**Deuterium Isotope Effects as Criteria of Mechanism in the Reactions of Organophosphorus Compounds**—D. SAMUEL, *Weizmann Institute of Science, Israel*

*Question:* Did you compare spontaneous acid hydrolysis with the analogous enzymic process with regard to the deuterium isotope effect?

I should also like to ask if, in your studies on D-labelled esters, you studied pH values other than pH 4? H. AEBI. *Berne University, Switzerland*

*Answer:* We have not done any enzymic studies on phosphate esters. Dr C. A. Vernon of University College, London, is working with various phosphatases, but I do not know whether he has investigated any deuterium isotope effects.

As to your second question, we have measured the kinetics of hydrolysis of various esters and other derivatives of phosphoric acid at various pHs. Only under certain conditions, such as in the case of the reactions of the monoanion at pH 4, is the reacting species sufficiently well-defined to allow a comparison to be made between $D_2O$ and $H_2O$. At other pHs, the variations in equilibrium constants and the mixture of species present prevent sufficiently accurate measurements from being made. SAMUEL

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*Question:* In connection with Dr Aebi's reference to biological systems, it should be noted that Garattini has shown that infant rat brain converts mevalonic acid to cholesterol more rapidly than it does mevalonic pyrophosphate, so that a prior hydrolysis obviously takes place. Since only the L form of mevalonic acid is biologically active, perhaps you should also consider optically active compounds in addition to the simpler analogues which you have been studying? D. KRITCHEVSKY, *Wistar Institute of Anatomy and Biology, U.S.A.*

*Answer:* I agree that the reactions of mevalonic acid phosphate itself should be investigated, and we hope to be able to do so as soon as sufficient quantities of this compound for kinetic studies can be prepared. SAMUEL

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**Kinetic Deuterium Isotope Effects in Enzymic Formate Oxidation**—H. AEBI, *Berne University, Switzerland*

*Comment:* It seems that the enzymic mechanism and the enzyme kinetics

presented by Dr Aebi are perhaps a little too oversimplified for the actual reaction studied and that it would, in fact, be very difficult to determine exactly where the isotope effect occurs. However, the observed changes in $V_{max.}$ could be taken to indicate an effect on the rate constant for the conversion of enzyme–substrate complex to product. Similarly, the observed changes in activation energy could indicate an effect on the rate constant for the formation of the enzyme–substrate complex. A change in $K$ seems more difficult to interpret. The reaction studied is irreversible, and it would perhaps be of interest to carry out similar experiments with a reversible system, since one could then investigate the rate of exchange at equilibrium, which would make it possible to determine isotope effects on specific rate constants. I wonder if anybody would care to comment on any work that might have been carried out on the subject. ERICSON

*Reply:* I agree with Dr Ericson. Furthermore, there is no reason why the approximations usually made in the kinetic treatment of enzyme reactions should not be applied in the two cases presented. The possible error introduced would be of quite different order of magnitude in comparison with the deuterium isotope effects observed. I should like to point out that several detailed investigations on reversible reactions have been made so far, *e.g.* by Dr R. Alberty, University of Wisconsin, in the case of fumarase. The result of an investigation on alcohol dehydrogenase, using deuterated substrate, was presented by Dr V. J. Shiner at the Symposium on Deuterium Isotope Effects organized by the New York Academy of Sciences in 1960.

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**Isotope Effects in Fully Deuterated Hexoses, Proteins and Nucleic Acids**—H. C. CRESPI *et al.*, *Argonne National Laboratory, U.S.A.*

*Comment:* We observed an interesting isotope effect in photosynthesis experiments with chlorella algae in HOT. After photosynthesis for a period of 20 minutes, the glucose produced from starch has no tritium at C–2. In other C atoms at least 10 per cent of the total intramolecular tritium is fixed. We assume that the reason for this is that the conversion of fructose-6-phosphate to glucose-6-phosphate involves an exclusive intramolecular proton transfer. Due to the stereospecificity of this reaction, only one hydrogen molecule of the fructose can be transferred. When this hydrogen is tritium an isotope effect must be expected, so that the fructose may tend to contribute to the transketolase reaction. The glucose from glucose-6-phosphate and sucrose has tritium at C–2. H. SIMON, *Technische Hochschule, Munich, Germany*

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*Question:* Have you ascertained whether flashing lights may be used to reduce the cell-division inhibition of growing plants by $D_2O$?

Does the temperature dependency of phycocyanin degradation vary with time? Were measurements made of the polarization of fluorescence

of the phycocyanin under these conditions? S. Aronoff, *Iowa State University, U.S.A.*

*Answer:* We have done no experiments with flashing lights, but it is possible that this procedure could lessen the degree of inhibition of division by $D_2O$.

As to your second question, below the critical temperature the fluorescence intensity is stable with time; above the critical temperature, degradation is continuous in time. No polarization measurements were made. Crespi

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*Question:* I should like to ask two questions regarding the biological properties of fully deuterated proteins. Does your statement that deuterated phycocyanin is hydrolysed by $\alpha$-chymotrypsin at a higher rate also hold for other proteolytic enzymes acting at a different pH, such as pepsin or catepsin?

Secondly, what are the antigenic properties of fully deuterated proteins, and is there any evidence to suggest that there is antigenic identity or non-identity between the "normal" protein and the corresponding deutero-protein? H. Aebi, *Berne University, Switzerland*

*Answer:* We began to study enzymes only recently and the only conclusion we have reached so far is that we shall not be able to isolate fully deuterated ribonuclease. However, we are now experimenting with other enzymes. We are also becoming convinced that the ordinary deuteroproteins are in fact one substance, so that we are not measuring a difference in, say, one amino-acid.

As to your question concerning antigenic properties, some preliminary experiments have been done which indicate that there may be some differences between deuterated and "normal" proteins. We have not been able, however, to reach any firm conclusions due to the difficulty in ensuring absolute purity. Crespi

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*Comment:* The reason why mammals cannot withstand high levels of deuteration, as indicated in the first slide Dr Crespi showed during his oral presentation, is not clear. In experiments carried out by my co-workers and myself on rats fed on 30 per cent $D_2O$ we, like other research workers, observed retarded growth and severe neurological symptoms. The animals then show signs of essential fatty acid deficiency and dermal lesions are found on their feet and tails. Preliminary gas-chromatographic analysis of the fatty acids of the brains, livers, hearts and spleens of $D_2O$-fed (30 per cent) rats and normal rats shows some differences in saturated/unsaturated fatty acid ratios. D. Kritchevsky, *Wistar Institute of Anatomy and Biology, U.S.A.*

*Comment:* I should like to refer to the effect of heavy water on cell growth and division. Clearly, the depression of "growth" becomes much more

severe, the higher the position of the organism in the phylogenetic scale, but this really corresponds, in large part, to a direct and rapid effect on cell division. One result of this effect is, in turn, an inhibition of DNA synthesis. This has now been observed in several types of higher plant and animal cells. One of my colleagues, Dr Bal, has, for instance, recently found that mitosis in onion root meristem, which stops within one intermitotic time in $D_2O$ media, is actually blocked at $G_1$, the pre-DNA synthetic gap. It is interesting to note that this is not found in many micro-organisms, as is shown by the fact that Drs Crespi, Katz and their collaborators were able to culture them in pure heavy water. The reason for this discontinuity is of interest and I am referring to it in my paper†. P. R. GROSS, *Brown University, U.S.A.*

*Reply:* With regard to Dr Kritchevsky's comment, I should like to point out that, in the case of rats to which 25 or 50 per cent $D_2O$ has been given in drinking water, there are significant changes in the enzyme pattern of liver and kidney tissue, *e.g.* catalase activity is reduced to a greater extent (−35 per cent) than the other enzymes tested. This indicates that there are also qualitative alterations in metabolism. On the other hand, the total oxygen consumption of liver and kidney slices from normal rats is affected only very little if $H_2O$ is replaced by $D_2O$ in the suspending medium. Both findings are compatible with the statement that the impairment of synthetic processes (proteins, DNA, RNA) must be considered as the main cause of the symptoms observed in $D_2O$-fed rats. AEBI

† These proceedings, *Pure Appl. Chem.* **8**, 483 (1964).