in addition to undergoing reactions (3b), $C_6H_5S\cdot$ enters into two termination processes:

$$C_6H_5S\cdot + (C_6H_5)_2CH\cdot \longrightarrow (C_6H_5)_2CHSC_6H_5 \text{ (V)} \quad (4a)$$
$$2\ C_6H_5S\cdot \longrightarrow C_6H_5SSC_6H_5 \text{ (VI)} \quad (4b)$$

The infrared spectrum of residues after isolation of III was identical with that of a solution prepared from an authentic sample of V[6] and VI.[7] Evidence for the occurrence of reaction (3b) was found in the formation of tetraphenylethane III in 72% yield by exposure of a dilute solution of diphenyl disulfide VI in diphenylmethane to a sun lamp. Studies with $S^{35}$ have shown that disulfides dissociate into mercaptyl radicals on irradiation.[8] Blank experiments indicated that compounds IV, V and VI did not cause formation of III from II, nor did they adversely affect the isolation of III from II.[9]

(6) C. Fingi and V. Bellavita, *Gazz. chim. ital.*, **62**, 699 (1932).
(7) T. Zinke and W. Frohneberg, *Ber.*, **43**, 840 (1910).
(8) E. N. Guryanova and V. N. Vasileva, *Zhur. Fiz. Khim.*, **28**, 60 (1954).
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## CONCERNING THE STEREOSPECIFICITY OF THE FUMARASE REACTION AND THE DEMONSTRATION OF A NEW INTERMEDIATE[1]

*Sir:*

When fumarase catalyzes the addition of water to fumarate only the L-isomer of malate is produced.[2] While the hydroxyl group is added stereospecifically, the question still arises as to the stereospecificity of the addition of the hydrogen atom. We have been able to demonstrate that this process is absolutely stereospecific.[3] Dipotassium fumarate was added to a reaction mixture containing crystalline fumarase,[4] 0.038 *M* $K_2HPO_4$, and 0.015 *M* $KH_2PO_4$ in a medium of 99.5% $D_2O$ at 25°. After equilibration it was found that the resulting L-malate, isolated as diphenacyl-L-malate, containing 0.97 excess atom of deuterium per molecule after exhaustive washing with water. The fumaric acid isolated had incorporated less than $1 \times 10^{-4}$ atom of non-exchangeable deuterium per molecule. This shows that the entering hydrogen atom is added in only one of the two possible positions and that a hydrogen atom from the identical position is removed in the dehydration reaction.

The stereospecificity with regard to the hydrogen atom having been established, the question as to whether the hydrogen and hydroxyl groups are added to fumarate in a *cis* or *trans* manner is currently being investigated in this laboratory.

The availability of the particular diamer of monodeutero-L-malate which is produced enzymatically made it possible to determine whether the breaking of the methylene carbon–hydrogen bond is the rate determining step in the enzymatic reaction. The breaking of the methylene carbon–deuterium bond in deutero-L-malate must proceed at approximately one-sixth the rate of that for the corresponding carbon–hydrogen bond.[5] If the breaking of a carbon–hydrogen bond were rate limiting, the over-all rate of reaction would be decreased by a factor of six in the dehydration of deutero-L-malate.

When monodeutero-L-malate was dehydrated enzymatically at *p*H 8.0 in 0.005 *M* tris-(hydroxymethyl)-aminomethane perchlorate buffer it was found that both the maximum initial velocity and Michaelis constant were unchanged after a correction for a small amount of fumarate in the malate preparation. Thus the breaking of the carbon–hydrogen bond is not involved in the rate-determining step. It seems likely from kinetic arguments[6,7] that the dissociation of fumarate from the enzyme is not the rate-determining step for the dehydration reaction at high L-malate concentrations.

The fact that the deuterium of monodeutero-L-malate was removed in a step which is not rate limiting suggested that there should be a sterically specific exchange of this hydrogen atom proceeding at a rate faster than the dehydration reaction. Such a rapid exchange was indeed found to occur in an experiment in which L-malate was dehydrated enzymatically in 99.5% $D_2O$ to the extent of 0.04%. The amount of deutero-L-malate which could have been formed by the reverse reaction from the fumarate so produced and initially present in the sample would be immeasurably small. However, the dipotassium malate recovered had incorporated about 0.003 atom of deuterium per molecule. This relatively rapid exchange demonstrates the existence of an intermediate in which the hydrogen atom has been removed from the methylene carbon of L-malate and which may be converted either into the enzyme–fumarate or enzyme–L-malate complex.

(5) F. H. Westheimer and N. Nicolaides, *ibid.*, **71**, 25 (1949).
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(7) R. A. Alberty, *J. Cellular Comp. Physiol.*, in press.

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(2) H. D. Dakin, *J. Biol. Chem.*, **52**, 183 (1922).
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## THE ADRENAL HORMONES AND RELATED COMPOUNDS. I. A "DIRECT" SYNTHESIS OF HYDROCORTISONE ACETATE AND CORTISONE ACETATE FROM 11α-HYDROXYPROGESTERONE

*Sir:*

The microbiological oxidation of progesterone described by Peterson, Murray, *et al.*,[1] provides an elegant method for the synthesis of 11α-hydroxyprogesterone (I) in high yield. The synthesis of

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