whose activity can be restored by addition of either FAD or the prosthetic flavin. (IIg) catalyzes the oxidation of acyl CoA's from C_3 to C_8. Setting the rate with BuCoA as 100 the respective rates for C_3, C_5, C_6, C_7, C_8, and C_{10} acyl CoA are 25, 55, 45, 35, 10 and 0. At the highest purity level (IIg) catalyzes the reduction of 200 μmoles of indophenol/min./μmole of bound flavin at 22°. The product of the oxidation of BuCoA by indophenol in presence of (IIg) has been identified as butenoyl CoA since it is not acted upon by (IV) (specific for β-hydroxyacyl CoA's) except in presence of the hydrase (III). Solutions of (IIg) are bleached within three seconds by BuCoA or instantaneously by dithionite. The leuco enzyme can be reoxidized by crotonyl CoA. The E_0' of the system BuCoA $\overset{-2E}{\rightleftarrows}$ crotonyl CoA lies in the range of indophenol (*ca.* + 0.2 v. at *p*H 7.0).

A flavoprotein (IIf), different and readily separable from (IIg) has been isolated from beef liver mitochondria and shown to catalyze only the oxidation of acyl CoA's with chain length > C_6.

Purified preparations of (III) have been obtained free of (I, II, IV and V) which catalyze the reactions

$$\text{RCH=CHCH}_2\text{COSCoA} \overset{\text{H}_2\text{O}}{\rightleftarrows} d\text{-RCH}_2\text{CHOHCH}_2\text{COSCoA} \overset{\text{H}_2\text{O}}{\rightleftarrows} \text{RCH}_2\text{CH=CHCOSCoA} \quad (2)$$

(III) acts upon all unsaturated acyl CoA's tested from C_4 to C_{12}. At the highest purity level, 1 mg. catalyzes the hydration of 500 μmoles of crotonyl CoA to *d*-β-hydroxybutyryl CoA per min. at 22°. At *p*H 9.0 the equilibrium ratio unsaturated:β-hydroxyacyl CoA lies between 0.5 and 1. (III) is not active on *cis*-crotonyl CoA. The isomerization of the *cis*- and *trans*-forms appears to be catalyzed by a separate enzyme. (III) is inhibited by sulfhydryl reagents.

The oxidizing enzyme[4] (IV) has been isolated without contamination by (I–III, V). It catalyzes the reaction

$$d\text{-RCHOHCH}_2\text{COSCoA} + \text{DPN}^+ \rightleftarrows \text{RCOCH}_2\text{COSCoA} + \text{DPNH} + \text{H}^+ \quad (3)$$

All hydroxyacyl CoA's from C_4 to C_{12} which have been tested are oxidized at approximately the same rate. At the highest purity level 1 mg. catalyzes the oxidation of 200 μmoles of β-hydroxyhexanoyl CoA per min. at 22° and at *p*H 9. DPN can be replaced by coenzyme III[14] but not by TPN. The enzyme is optically specific for the product of the hydrase reaction, *i.e.*, *d*-β-hydroxyacyl CoA.[15] The E_0' for the reaction has been found to be −0.224 v.[16] The products of oxidation of the C_4, C_6 and C_8 β-hydroxyacyl derivatives of CoA were isolated and identified as the β-ketoacyl derivatives by chemical, enzymatic and optical methods.[17]

(V)[4,5,6] which has been separated from the other enzymatic components catalyzes the reaction

$$\text{RCOCH}_2\text{COSCoA} + \text{CoA} \rightleftarrows \text{RCOSCoA} + \text{AcCoA} \quad (4)$$

The same enzyme appears to be active on all β-ketoacyl CoA derivatives regardless of chain length, at least from C_4 to C_{12}. At the highest purity level 1 mg. of (V) catalyzes the cleavage of 10 μmoles of β-ketohexanoyl CoA per min. at 30° and *p*H 7.7. The products of the cleavage of β-ketohexanoyl CoA have been identified as BuCoA and AcCoA.

All the enzymatic steps of fatty acid oxidation have been shown to be reversible. The enzymatic synthesis of BuCoA in high yield from AcCoA has now been demonstrated. For this synthesis IIg and reduced DPN and benzyl viologen are necessary. BuCoA was identified as Bu hydroxamic acid after chromatographic separation from other acyl derivatives.[18]

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RECEIVED MAY 15, 1953

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[14] T. P. Singer and E. B. Kearney, *Biochim. et Biophys. Acta*, **8**, 700 (1952).

[15] A. L. Lehninger and G. D. Greville, THIS JOURNAL, **75**, 1515 (1953).

[16] 0.320 v. was used as the E_0' for the DPN couple; K. Burton and T. H. Wilson, *Biochem. J.*, **54**, 98 (1953).

[17] H. Beinert, *J. Biol. Chem.*, in press.

[18] Since this manuscript was first submitted for review on April 8, 1953, communications have appeared in THIS JOURNAL by Stern and del Campillo (**75**, 2277 (1953)), and by Seubert and Lynen (**75**, 2787 (1953)) on aspects of fatty acid oxidation.

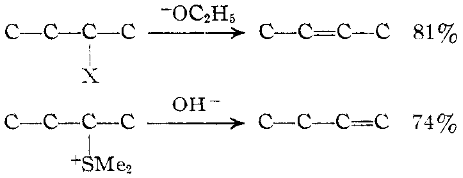
[19] Supported by a grant from the National Heart Institute of the National Institutes of Health.

[20] Supported by a grant-in-aid of the American Cancer Society (on recommendation by the committee on Growth, National Research Council).

A NEW TECHNIQUE FOR CONTROLLING THE DIRECTION OF ELIMINATION REACTIONS

Sir:

It has been maintained by Ingold and his coworkers [Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter VIII] that bimolecular eliminations from alkyl halides result in the predominant formation of the most highly branched olefin (Saytzeff rule), whereas onium salts give the least branched olefins (Hofmann rule).



$$\underset{\displaystyle\text{X}}{\text{C—C—C—C}} \xrightarrow{^-\text{OC}_2\text{H}_5} \text{C—C=C—C} \quad 81\%$$

$$\underset{\displaystyle ^+\text{SMe}_2}{\text{C—C—C—C}} \xrightarrow{\text{OH}^-} \text{C—C—C=C} \quad 74\%$$

They have attributed the change in direction of elimination to the inductive effect of the positive pole in the onium salt. Schramm [C. H. Schramm, *Science*, **112**, 367 (1950)] suggested that the effect might be due not to the charge, but to the large steric requirements of the dimethylsulfonium or trimethylammonium group which would favor attack by the base on a terminal hydrogen atom.

We had previously observed that the unimolecular elimination of diisobutylene hydrochloride proceeds to give predominantly the 1-olefin and attributed this result to steric effects [H. C. Brown and H. L. Berneis, THIS JOURNAL, **75**, 10 (1953)]. We were therefore led to consider the possibility that steric effects might be made to control the direction of elimination and that such steric effects might be the structural basis for eliminations according to the Hofmann rule.

We have now found it possible to control the direction of elimination in alkyl halides by varying the steric requirements of the attacking base. The use of potassium *t*-butoxide gives predominantly the 1-olefin in cases where the ethoxide results in the 2-derivative.

Alkyl halide	% 1-Olefin	
	EtO^-	*t*-BuO^-
$C_2H_5CHBrCH_3$	19 (ref. 1)	53.4
$C_3H_7CHBrCH_3$	29 (ref. 1)	66
$C_2H_5CBr(CH_3)_2$	29 (ref. 1)	72
$(CH_3)_2CHCBr(CH_3)_2$		87
$(CH_3)_3CCH_2CBr(CH_3)_2$	85	99

Bases with larger steric requirements result in a further increase of the 1-olefin. This may be illustrated by the increasing yields of 3-methyl-1-butene which are obtained from *t*-amyl bromide in bimolecular eliminations utilizing a series of alkoxides of increasing steric requirements.

Potassium salt of	% 1-Olefin
Ethyl alcohol	29
t-Butyl alcohol	72
t-Amyl alcohol	78
Triethylcarbinol	89

Sufficient potassium metal was dissolved in 200 ml. of the alcohol to give a solution approximately 1.5 *M* in the alcoholate. The *t*-halide was then dissolved in the solution, maintaining a 50% molar excess of base. The solution was heated at 75° for two hours to ensure completion of the reaction. The temperature was then raised and the olefin distilled out of the reaction mixture through an efficient micro column. Olefin yields (based on *t*-halide) of 93–99% were obtained. The products were analyzed by refractive index and checked in selected test cases by infrared analysis.

The use of potassium *t*-butoxide and other even more hindered bases should be a valuable synthetic tool in controlling the direction of elimination and should do away with the need to synthesize quaternary ammonium compounds in order to obtain high yields of terminal olefins.

We have now been able to demonstrate a general trend from elimination according to the Saytzeff rule toward elimination according to the Hofmann rule by (1) increasing the steric requirements of the alkyl groups on the incipient double bond ($Me_2<Me, Et<Me_3<Me_4<Me_2$, *t*-Bu), (2) increasing the steric requirements of the group undergoing elimination ($Br^-<{}^-OSO_2R<SMe_2<NMe_3$), and (3) increasing the steric requirements of the attacking base ($EtO^-<$*t*-BuO^-).

These results leave little doubt that steric effect must be the basis of eliminations according to the Hofmann rule.

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RECEIVED JULY 20, 1953

NUCLEOTIDE SYNTHESIS BY MALT AND PROSTATE PHOSPHATASES

Sir:

In an extension of previous work[1] on the phosphorylation of nucleosides by phosphate transfer we have made a search for other transfer systems. As regards the malt enzyme used in the previous experiments, the mononucleotides themselves have been found to be much more efficient donors, as judged by the transfer ratio,[2] than sodium phenylphosphate employed previously.[1] Deoxy- and ribonucleotides, but only the 5′-isomers, were equally effective as donors.

It has, in addition, been found that human prostate phosphatase also is able to catalyze the phosphorylation of nucleosides and that in this case, in contrast to the malt enzyme, which produces only the 5′-nucleotides, all possible nucleotide isomers are formed. In the prostate enzyme system, phenylphosphate served as a donor, but mononucleotides did not. These differences in specificity are summarized below.

		Nucleotides synthesized				
Enzyme	Donor	5′-ribo	3′-ribo	2′-ribo	5′-deoxy	3′-deoxy
Malt	Phenylphosphate	+	−	−	+	−
	Mononucleotide	+	−	−	+	−
Prostate	Phenylphosphate	+	+	+	+	+
	Mononucleotide	−	−	−	−	−

With the use of the prostate enzyme and of phenylphosphate as donor, the three isomers of ribocytidylic acid and the two isomers of thymidylic acid have been synthesized and isolated by ion exchange chromatography.

This work was supported by research grants from the National Institutes of Health, U. S. Public Health Service, and the Rockefeller Foundation. One of us (G. B.) was aided by a Predoctoral Research Fellowship from the U. S. Public Health Service.

(1) G. Brawerman and E. Chargaff, THIS JOURNAL, **75**, 2020 (1953).

(2) B. Axelrod, *J. Biol. Chem.*, **172**, 1 (1948).

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RECEIVED JULY 6, 1953

THE INFRARED SPECTRUM OF THE OXONIUM ION

Sir:

The infrared absorption spectra of films of oxonium chloride and oxonium bromide have been observed at −195° and spectra which are typical of those obtained are reproduced in Fig. 1. The films were prepared by condensing an equimolar mixture of gaseous H_2O and HX on a previously cooled KBr plate. The OH_3^+ must be the source of the four absorption bands at 1050 cm.$^{-1}$, 1700 cm.$^{-1}$, 2100 cm.$^{-1}$ and 2570 cm.$^{-1}$ in OH_3Cl (similarly, at 1100 cm.$^{-1}$, 1700 cm.$^{-1}$, 2100 cm.$^{-1}$ and 2610 cm.$^{-1}$ in OH_3Br). In addition, some films of