sion to dl-$\Delta^{9(11),16}$-21-norprogesterone previously prepared by Woodward.[1]

The acetonide was converted in excellent yield to dl - 3 - keto - 11β,16β,17β - trihydroxy - Δ^4 - 9α-bromo-D-homoandrostene acetonide (m.p. 194–196°) with N-bromosuccinimide and sulfuric acid in aqueous acetone.[6] The crude bromohydrin was converted by alkali to dl-3-keto-9β,11β-oxido-16β,17β-dihydroxy-Δ^4-D-homoandrostene acetonide (m.p. 191–193°. Found: C, 74.3; H, 8.7). This crude bromohydrin was also oxidized with pyridine–chromium trioxide complex[7] to give a crude bromoketone (m.p. 195–198° dec.) which without purification was debrominated with zinc and aqueous acetic acid to give dl-3,11-diketo-Δ^4-16β,17β-dihydroxy-D-homoandrostene acetonide (m.p. 198–200°). Treatment with periodic acid followed by benzene and piperidine acetate[1] gave dl-11-keto-Δ^{16}-21-norprogesterone III (m.p. 207–209°. Found: C, 76.5; H, 7.7). Reaction with alkaline hydrogen peroxide[8] produced dl-11-keto-16α,17α-oxido-21-norprogesterone (m.p. 243–245°). Oxidation with silver oxide gave dl-3,11-diketo-16α,-17α-oxido-Δ^4-etiocholenic acid (m.p. 217–220° dec.). Reaction of the dry sodium salt with oxalyl chloride yielded an acid chloride which on treatment with diazomethane[9] gave a crystalline diazoketone (m.p. 193–195°) having strong infrared absorption at 4.75μ. Reaction of the diazoketone with hot acetic acid gave non-crystalline dl-16α,-17α - oxido - 3,11,20 - triketo - 21 - hydroxy - Δ^4-pregnene acetate. Opening with hydrogen bromide[8] produced dl-16β-bromocortisone acetate (m.p. 238–240° dec.). Debromination with Raney nickel[8] gave dl-cortisone acetate[10] (m.p. 240–243°) whose infrared spectrum was identical with natural cortisone acetate.

We thank Dr. R. H. Munch, Mr. G. W. Ashworth and Mr. O. E. Kinast for help with the numerous infrared and ultraviolet spectra needed in this work. In addition, we acknowledge the invaluable advice and assistance of Dr. R. B. Woodward.

(6) After the completion of our work, J. Fried and E. F. Sabo [THIS JOURNAL, **75**, 2273 (1953)] reported that they added hypobromous acid in good yield to a 3-keto-$\Delta^{4,9(11)}$ steroid. It now appears that the low yield obtained by Hicks and Wallis [*J. Biol. Chem.*, **162**, 641 (1946)] may be attributed to the fact that in their case rings A and B were *cis*.

(7) A reagent first announced at the Gordon Research Conferences, A.A.A.S., New Hampton, N. H., August 4–8, 1952; *cf.* G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(8) *Cf.* P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller, *ibid.*, **72**, 5145 (1950).

(9) *Cf.* A. L. Wilds and C. H. Shunk, *ibid.*, **70**, 2427 (1948).

(10) L. H. Sarett, G. E. Arth, R. M. Lukes, R. E. Beyler, G. I. Poos, W. F. Johns and J. M. Constantin, *ibid.*, **74**, 4974 (1952).

ORGANIC CHEMICALS DIVISION
ST. LOUIS RESEARCH DEPARTMENT
MONSANTO CHEMICAL COMPANY
ST. LOUIS, MISSOURI

LLOYD B. BARKLEY
MARTIN W. FARRAR
WILLIAM S. KNOWLES
HAROLD RAFFELSON

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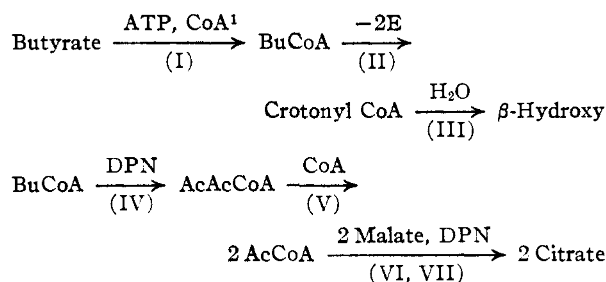
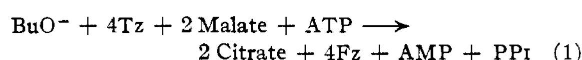
THE RECONSTRUCTION OF THE FATTY ACID OXIDIZING SYSTEM OF ANIMAL TISSUES

Sir:

A system including seven enzymes has been shown to catalyze the following sequence



where (I) represents the fatty acid activating enzyme,[2,3] (II) fatty acyl CoA dehydrogenase, (III) unsaturated acyl CoA hydrase, (IV) β-hydroxyacyl CoA dehydrogenase,[4] (V) AcAcCoA cleavage enzyme,[4,5,6] (VI) malic dehydrogenase[7] and (VII) AcCoA-oxalacetate condensing enzyme.[8] Enzymes (I–V) have been isolated from beef liver mitochondria. Tz is the final electron acceptor with pyocyanine as intermediary carrier. Diaphorase[9] (VIII) catalyzes the oxidation of DPNH. The over-all balanced reaction is



$$BuO^- + 4Tz + 2\,Malate + ATP \longrightarrow 2\,Citrate + 4Fz + AMP + PP_I \quad (1)$$

The observed citrate:Fz ratio of 1:2.2 is in good agreement with the 1:2 ratio of equation (1).

Preparations of (I) at the highest purity level are homogeneous in the ultracentrifuge. At pH 10 with heptanoate as substrate, 1 mg. of (I) catalyzes the formation of 3.8 μ mole of acyl CoA per min. at 38°. (I) activates a wide variety of odd or even, straight (C_4–C_{12}), branched chain, or substituted fatty acids as well as α,β- and β,γ-unsaturated acids. (I) has proved invaluable for preparation of all acyl CoA derivatives required as substrates for (II–IV). The mechanism of activation by ATP is the same as for the acetate activation enzyme system.[10]

(IIg) a green copper flavoprotein[11] has been isolated in a form which is homogeneous in both the ultracentrifuge and Tiselius apparatus. The riboflavin content of the homogeneous enzyme is 1.2%. The prosthetic flavin has the same absorption spectrum and enzymatic activity as FAD.[12,13] (IIg) can be converted into an apoenzyme at pH 3.7

(1) The following abbreviations will be used: adenosinetriphosphate (ATP); adenosine-5'-phosphate (AMP); coenzyme A (CoA); di- and triphosphopyridine nucleotide (DPN, DPNH and TPN, TPNH); flavin adenine dinucleotide (FAD); acetyl (Ac); acetoacetyl (AcAc); butyryl (Bu); triphenyltetrazolium (Tz); formazan (Fz); and inorganic pyrophosphate (PP_I).

(2) H. R. Mahler, "Phosphorus Metabolism," Vol. 2, 286, Johns Hopkins Press, Baltimore, 1953; H. R. Mahler, S. J. Wakil and R. M. Bock, *J. Biol. Chem.*, in press.

(3) G. Drysdale and H. A. Lardy, "Phosphorus Metabolism," Vol. 2, p. 281, Johns Hopkins Press, Baltimore, Md., 1953.

(4) F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

(5) J. R. Stern, M. J. Coon and A. del Campillo, *Nature*, **171**, 28 (1953).

(6) D. E. Green, D. S. Goldman, S. Mii and H. Beinert, *J. Biol. Chem.*, **202**, 137 (1953).

(7) F. B. Straub, *Z. physiol. Chem.*, **275**, 63 (1942).

(8) S. Ochoa, J. R. Stern and M. C. Schneider, *J. Biol. Chem.*, **193**, 691 (1951).

(9) J. G. Dewan and D. E. Green, *Biochem. J.*, **32**, 626 (1938).

(10) H. Beinert, D. E. Green, P. Hele, H. Hift, R. W. Von Korff and C. V. Ramakrishnan, *J. Biol. Chem.*, **203**, 35 (1953).

(11) H. R. Mahler, THIS JOURNAL, **75**, 3288 (1953).

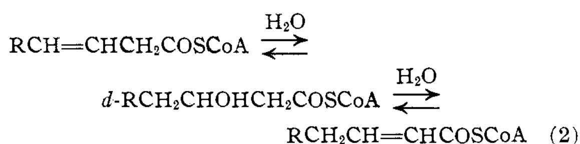
(12) E. Negelein and H. Brömel, *Biochem. Z.*, **300**, 225 (1939).

(13) O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150 (1938).

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 $\xrightleftharpoons{-2E}$ 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.

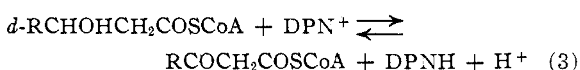
Purified preparations of (III) have been obtained free of (I, II, IV and V) which catalyze the reactions



$$RCH{=}CHCH_2COSCoA \xrightleftharpoons{H_2O} d\text{-}RCH_2CHOHCH_2COSCoA \xrightleftharpoons{H_2O} RCH_2CH{=}CHCOSCoA \quad (2)$$

(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



$$d\text{-}RCHOHCH_2COSCoA + DPN^+ \rightleftharpoons RCOCH_2COSCoA + DPNH + H^+ \quad (3)$$

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]

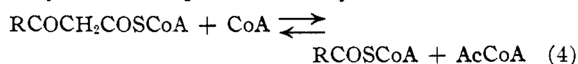
(14) T. P. Singer and E. B. Kearney, *Biochim. et Biophys. Acta*, **8**, 700 (1952).

(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.

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



$$RCOCH_2COSCoA + CoA \rightleftharpoons RCOSCoA + AcCoA \quad (4)$$

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]

INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

HELMUT BEINERT
R. M. BOCK
DEXTER S. GOLDMAN
D. E. GREEN[19]
H. R. MAHLER[20]
SANAE MII
P. G. STANSLY
S. J. WAKIL

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(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.

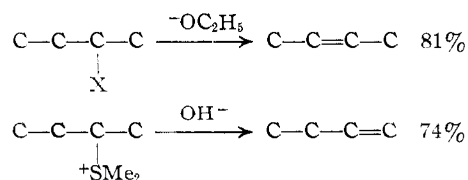
(19) Supported by a grant from the National Heart Institute of the National Institutes of Health.

(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).



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