TABLE I

SUBSTRATES FOR THIOESTERASE

15-fold purified chicken liver thioesterase. ~0.5 μM thioester in 0.067 M Tris-HCl buffer pH 7.0. Specific activity = $-\Delta E_{240}$ per minute per mg. protein. Values in parentheses refer to synthetic S-acetyl ester.

	Specific activity
8-S-AcAc-MTO	82
(±)-8-S-AcAc,6-ethyl-MTO	69
(±)-S-AcAc-DTO	56 (6.0)[a]
(±)-6-S-AcAc-MTO	17
(±)-6-S-AcAc-Decanoate	7.5
S-AcAc-BAL	310 (30)

[a] (+)-6-S-Ac-DTO was hydrolyzed at same rate as the synthetic ester.

thioester and *one* mole of thiol are formed. This stoichiometry is consistent with reaction 3 and follows from the fact that AcAc-SR does not assay as thioester by the hydroxylamine method[5] while S-Ac-DTO assays as both thiol and thioester. The Ac-S-Pn and Ac-S-DTO formed were further identified by paper chromatography. It is not yet determined whether 6- or 8-S-Ac-DTO is formed. This mixed thiolysis reaction represents a novel enzymatic synthesis of S-Ac-DTO. It differs from the synthesis of (+)-6-S-Ac-DTO from Ac-S-CoA and (−)-DTO catalyzed by DTO transacetylase[7,8] in that it involves transacetylation with a 4-carbon fragment and utilizes both (−)-DTO and (+)-DTO.[13] The enzyme(s) catalyzing reaction 3 differs from the thiolase[14,15] of the fatty acid cycle (reaction 1) in that (a) AcAc-S-Pn is more reactive than AcAc-S-CoA, (b) it is less sensitive to iodoacetamide, and (c) it is not readily reversible, if at all. Interestingly, other thiol compounds which activate AcAc synthesis (*e.g.*, BAL, GSH, cysteine) can substitute for DTO in reaction 3, yielding the corresponding S-Ac ester.

TABLE II

THIOLYSIS OF AcAc-S-Pn BY DTO

1.50 μM AcAc-S-Pn, 4.0 μM DTO, 150 μM Tris-HCl buffer pH 8.1 and 0.9 mg. ox liver protein. Incubated 13 minutes at 25°. Values in μM.

	Acetyl acceptor		
	(±)-DTO	(+)-DTO	(−)-DTO
Δ AcAc-S-Pn[a]	−1.50	−1.50	−1.50
Δ Sulfhydryl[b]	+1.43	+1.44	+1.71
Δ Hydroxamic acid	+2.99	+2.98	+2.88

[a] Measured optically at 310 mμ. [b] Nitroprusside assay.

(±)-S-AcAc-DTO is reduced by DPNH in the presence of crystalline heart β-hydroxybutyryl-S-CoA dehydrogenase.[16] Liver fractions do not catalyze a thiolysis of (±)-S-AcAc-DTO by DTO, CoA-SH, Pn-SH or GSH. Nor do they convert (+)-6-S-Ac-DTO[7] or (±)-8-S-Ac-DTO to AcAc.

Since thioesterases exist in liver for S-AcAc-DTO and AcAc-SG, the latter are possible intermediates in AcAc synthesis. However, experiments so far have failed to demonstrate their enzymatic formation, *e.g.*, by AcAc transfer from AcAc-S-CoA (or Pn). The precise role of DTO and other thiols in activating AcAc synthesis is being investigated further.

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(13) We are indebted to Dr. K. Folkers of Merck-Sharp and Dohme Research Laboratories for (+) and (−) lipoic acids.

(14) J. R. Stern and S. Ochoa, in "Biochemical Problems of Lipids," Butterworths Publications, London, 1956, p. 162.

(15) F. Lynen, K. Decker, O. Wieland and D. Reinwein, ref. 14, p. 142.

(16) J. R. Stern, unpublished experiments.

A NEW PATHWAY FOR PROPIONATE OXIDATION

Sir:

Oxidation of propionate in animal tissues occurs by a carboxylation pathway through methyl malonate to succinate.[1] Stadtman[2] has observed the formation of β-alanyl-CoA from acryl-CoA extracts of *Clostridium propionicum*. Mahler and Huennekens suggest an α-oxidative pathway.[3] In this communication evidence is presented in support of a β-oxidative pathway in peanut mitochondria.[4]

The oxidation of sodium propionate-1-C^{14} to $C^{14}O_2$ by mitochondria isolated from cotyledons of germinated peanuts[4a] is dependent upon ATP,[5] CoA, DPN, GSH and αKG; TPN and Mn^{++} stimulate the oxidation.

TABLE I

COFACTOR REQUIREMENTS FOR OXIDATION OF PROPIONATE-1-C^{14} TO $C^{14}O_2$

The complete reaction mixture contained 0.1 μmole propionate-1-C^{14} (5500 c.p.m.); 0.5 ml. mitochondria (approximately 20 mg. protein) in 0.2 M Tris- 0.5 M sucrose, pH 7.2, containing about 5×10^{-3} % BAL; 10 μmoles phosphate buffer, pH 7.1; 50 μmoles KCl; 1 μmole ATP; 0.3 μmole CoA; 0.2 μmole DPN; 0.1 μmole TPN; 5 μmoles GSH; 1 μmole αKG; 0.2 ml. 20% KOH in the center well; 0.3 ml. 10 M H_2SO_4 in the sidearm, final volume 1.7 ml. Time of incubation, 2 hr., temperature 25°. % oxidation = $BaC^{14}O_3$ (c.p.m.) × 100/substrate (c.p.m.).

Components	% Oxidation	Components	% Oxidation
Complete	24	−TPN	21
−ATP	1	−GSH	17
−CoA	2	$-MnSO_4$	20
−DPN	11	−αKG	5

Since pools of pyruvate, lactate, succinate and methyl malonate added during propionate-1-C^{14} oxidation do not acquire any label, these compounds do not appear to participate as intermediates. Furthermore, no propionate-dependent fixation of $C^{14}O_2$ can be demonstrated.

To examine the course of oxidation, propionate-1-C^{14}, -2-C^{14} and -3-C^{14}[6] were incubated with a complete reaction mixture for different periods of time and ether-extractable reaction products separated by paper chromatography. In each case a new radioactive spot (R_f 0.25 in ethanol ammonia; propionate R_f 0.42) appeared, which decreased

(1) M. Flavin, P. J. Ortiz and S. Ochoa, *Nature*, **176**, 823 (1955).

(2) E. R. Stadtman, *Federation Proc.*, **15**, 360 (1956).

(3) H. R. Mahler and F. M. Huennekens, *Biochim. et Biophys. Acta*, **11**, 575 (1953).

(4) In a recent personal communication, Dr. M. J. Coon describes a propionate → βHP → β-alanine pathway in animal tissues. In peanut mitochondria no β-alanine accumulates.

(4a) P. K. Stumpf, *Plant Physiology*, **30**, 55 (1955).

(5) Abbreviations: ATP, adenosine triphosphate; CoA, coenzyme A; DPN, diphosphopyridine nucleotide; GSH, glutathione; Mn^{++}, manganese; αKG, α-ketoglutarate; βHP, β-hydroxypropionate.

(6) Kindly donated by Dr. Harland G. Wood.

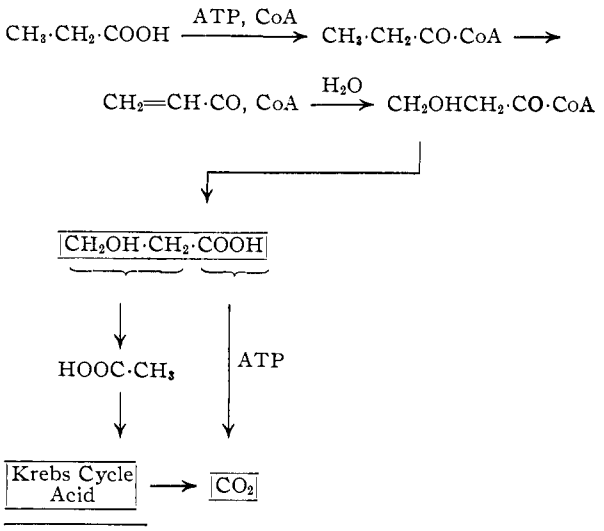
with time. This spot, identified as βHP, was characterized by (a) two dimensional co-chromatography with authentic βHP, (b) dichromate oxidation[7] of βHP derived from propionate-2-C^{14}, to a dicarboxylic acid which co-chromatographed with malonic acid. The dicarboxylic acid was degraded by pyrolysis[8] to radioactive acetate and CO_2 and (c) esterification of the acid with diazomethane and conversion to a hydroxamic acid (with hydroxylamine) which co-chromatographed with authentic βHP hydroxamate.

Both propionate-1-C^{14} and βHP-1-C^{14}[9] rapidly release the carboxyl-C^{14} as $C^{14}O_2$. No radioactive Krebs cycle acids are produced. The cofactor required for the oxidation of βHP-1-C^{14} to $C^{14}O_2$ is ATP.

Under the same conditions, propionate-2-C^{14} and βHP-2-C^{14}[9] slowly release C^{14} as $C^{14}O_2$, only after the appearance of radioactive citric, succinic, malic and fumaric acids. With propionate-3-C^{14} a rate of $C^{14}O_2$ release between that of propionate-1-C^{14} and of -2-C^{14} is observed with the formation of labeled Krebs cycle acids. Succinate derived from propionate-1-C^{14} is not labeled. Succinate derived from propionate-2-C^{14} is labeled exclusively in the methylene groups and succinate derived from propionate-3-C^{14} is labeled exclusively in the carboxyl groups.

The conversion of propionate-C^{14} to βHP-C^{14} is dependent on oxygen, ATP and CoA. Other cofactors were not tested.

The pathway may be formulated as[10]



(7) By the method used for lactate oxidation in S. Aronoff, "Techniques of Radiobiochemistry," The Iowa State College Press, Ames, Iowa, 1956, p. 141.

(8) S. Aronoff, ref. 7, p. 140.

(9) Radioactive βHP, presumably labeled as shown, was isolated by paper chromatography of the reaction product of propionate-1-C^{14} or -2-C^{14} oxidation.

(10) Compounds in blocks were isolated and characterized.

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THE STEREOSPECIFIC RADICAL ADDITION OF HYDROGEN BROMIDE TO *cis*- AND *trans*-2-BROMO-2-BUTENE[1]

Sir:

In previous work it was found that a high degree of stereospecificity is observed in the radical addition of hydrogen bromide to cyclohexene[2,3] and cyclopentene[4] derivatives. From this and other considerations it appeared possible that under favorable conditions stereospecificity might be observed with this addendum in acyclic systems. We wish to report that the radical addition of hydrogen bromide to the isomeric 2-bromo-2-butenes in liquid hydrogen bromide at −80° is indeed stereospecific and results in almost complete *trans* addition. Other radical additions which have been investigated have been found to be non-stereospecific[5] and apparently this is the first report of a stereospecific radical addition in an acyclic system.

The additions were promoted by irradiation with a quartz-jacketed Hanovia type SC-2537 lamp which fit into the reaction vessel so that the reaction mixture occupied the annular space between the walls of the lamp and reaction vessel. During the reaction the vessel was immersed in a Dry Ice–acetone bath. Mixtures of approximately 20 ml. of anhydrous liquid hydrogen bromide and 5 g. of *cis*- or *trans*-2-bromo-2-butene (shown to be homogeneous by gas chromatographic analysis[6] and physical properties) were irradiated after which the hydrogen bromide was allowed to evaporate and the composition of the residual reaction mixture determined by gas chromatographic[6] and infrared analysis.

In a typical experiment pure *cis*-2-bromo-2-butene in liquid hydrogen bromide was irradiated for 7.5 minutes. The composition of the product (gas chromatographic analysis[6]) was found to be < 0.5% 2-bromo-2-butene (largely isomerized to the *trans* isomer), 3% 2,2-dibromobutane (presumably formed by ionic addition), 92% *meso*-2,3-dibromobutane, 5% *dl*-2,3-dibromobutane and < 0.5% 1,2-dibromobutane.

Irradiation of *trans*-2-bromo-2-butene for 15 minutes under similar conditions gave a mixture consisting of 5% of unreacted *trans*-2-bromo-2-butene, 83% *dl*-2,3-dibromobutane, 8.5% *meso*-2,3-dibromobutane and 3.5% 1,2-dibromobutane. The analytical method was calibrated and confirmed using pure authentic samples of all of the components and synthetic mixtures of these. Infrared spectroscopic examination of the reaction products also showed that the *cis*-bromobutene is converted primarily to *meso*-dibromide and the

(1) This work was supported by the United States Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command under contract No. AF 18(600)1037.

(2) H. L. Goering, P. I. Abell and B. F. Aycock, Jr., THIS JOURNAL, **74**, 3588 (1952).

(3) H. L. Goering and L. L. Sims, *ibid.*, **77**, 3465 (1955).

(4) K. L. Howe, unpublished work.

(5) P. S. Skell and R. C. Woodworth, THIS JOURNAL, **77**, 4638 (1955); P. S. Skell, R. C. Woodworth and J. H. McNamara, *ibid.*, **79**, 1253 (1957).

(6) Excellent separation was obtained with a 10 ft. column packed with the commercial detergent, Tide. An operating temperature of 70° and flow rate of 40 ml. helium per minute were used to check for intercontamination of the isomeric 2-bromo-2-butenes. An operating temperature 120° was used for analysis of the reaction mixtures.