LIPOIC ACID DEHYDROGENASE: THE FUNCTION OF E. COLI FRACTION B*

Sir:

The oxidative decarboxylation of pyruvate by extracts of *Escherichia coli* is dependent on two enzyme fractions referred to as A and B which in the presence of pyruvate form acetyl-phosphate, provided transacetylase and orthophosphate are present, or citrate, provided condensing enzyme and oxaloacetate are present.[1] From knowledge of the reactions catalyzed by phosphotransacetylase[2] and the condensing enzyme[3] the function of *E. coli* fractions A and B were deduced as the catalysis of the reaction

$$\text{Pyruvate} + \text{DPT}^+ + \text{DPN}^+ + \text{CoA} \rightleftharpoons \text{Acetyl-CoA} + \text{CO}_2 + \text{DPNH} + \text{DPT}^+ \quad (1)$$

Subsequently further purified fractions A and B were shown to catalyze reaction 1 in the presence of substrate amounts of DPN and CoA.[4,5] Fraction A has been shown to contain the carboxylase enzyme; *i.e.*, catalyzes $C^{14}O_2$ exchange into pyruvate[6] in the presence of DPT, and the oxidative decarboxylation of pyruvate in the presence of DPT and ferricyanide[4] (Green[7], *et al.*, assay). Further evidence of the function of Fraction B was obtained[5] from its activation of the alpha-ketoglutarate carboxylase (Fraction A′) for DPN reduction in the presence of DPT and CoA according to the reaction

$$\alpha\text{-ketoglutarate} + \text{DPT}^+ + \text{DPN}^+ + \text{CoA} \rightleftharpoons \text{succinyl-CoA} + \text{CO}_2 + \text{DPNH} + \text{DPT}^+ \quad (2)$$

Since the transacetylase enzymes show a high degree of specificity and the only reaction common to all keto-acid dehydrogenases is the DPN reduction, it was suggested[4] that coli fraction B is the dehydrogenase which functions via the reoxidation of lipoic acid as follows

$$\text{Lip(SH)}_2 + \text{DPN}^+ \rightleftharpoons \text{LipS}_2 + \text{DPNH} + \text{H}^+ \quad (3)$$

Lipoic acid had previously been shown to be essential in alpha-keto acid and dicarbonyl cleavage by independent experiments using *Streptococcus faecalis*,[8] *Tetrahymena geleii*,[9] and more recently with a mutant of *E. coli*.[10] Lipoic acid was previously suggested to act[5] as acceptor for the "aldehyde-"[6] generated by keto acid decarboxylation with consequent oxidation to acyl, acetyl or succinyl of equations 1 and 2, to form thio esters of lipoic acid. The transfer of the acyl from lipoic acid to coenzyme A—by lipoic transacylases—would regenerate reduced lipoic acid which in turn would be reoxidized via DPN as electron acceptor (lipoic dehydrogenase). Reed and DeBusk[11] have outlined a reaction sequence similar to that given previously,[4] with the added suggestion that lipoic acid functions as lipothiamide pyrophosphate, the amide of lipoic acid with the 4-aminopyrimidyl group of thiamine.

Lipoic dehydrogenase can be measured directly by following DPN reduction (reaction 3) or more conveniently by following the disappearance of thiol groups in the presence of pyruvate, lactic dehydrogenase and catalytic amounts of DPN according to the reaction

$$\text{Lip(SH)}_2 + \text{Pyruvate} \xrightleftharpoons{\text{(DPN)}} \text{LipS}_2 + \text{Lactate} \quad (4)$$

The substrate and cofactor dependencies of reaction (4), measured by disappearance of thiol groups, is shown in Table I.

TABLE I

A DPN LINKED LIPOIC ACID DEHYDROGENASE

	Additions	Lip(SH)$_2$ Oxidation μM/30′
1.	Complete System[a]	7.7
2.	Complete System − DPN, 0.5 μM	0.4
3.	Complete System − Pyruvate, 20 μM	0.4
4.	Complete System − Lactic Dehydrogenase, 2000 U	0.2
5.	Complete System − Fraction B, 140 γ (S.A. 200[b] μM)	0.0

[a] Additions 2 through 5, plus; 10 μM reduced lipoic acid (Lip(SH)$_2$), 100 μM phosphate buffer, *p*H 6.0. [b] S.A. = μM lipoic acid oxidized/hr./mg. Protein.

Table II shows the lipoic dehydrogenase activity of fraction B over a 200-fold range of purity as compared to its activation of the dismutation reaction in the presence of excess fraction A, which contains lipoic acid. As the data show, the lipoic dehydrogenase activity of fraction B is approximately twice, (1.8×), its dismutation activity. A similar correlation with the rate of DPN reduction in the presence of enzymes A and B plus DPN, DPT and

TABLE II

PURIFICATION OF LIPOIC DEHYDROGENASE: COLI FRACTION B

	Fraction	Protein, g.	Lipoic DeH Sp. Act.[d]	Lip.[a] DeH × 10^3	Units of Pyr.[b] Dism. × 10^3	Ratio L/D
1.	Cell Extract	6.3	4.85	30.6	18	1.7
2.	1 + 25–75 $AmSO_4$[c]	6.0	3.21	19.1	10	1.82
3.	2 + Protamine	2.4	6.95	16.5	8.8	1.88
4.	3 + 60–100 $AmSO_4$	0.52	34.6	18.0	10.4	1.73
5.	4 + 50–60 $AmSO_4$	.15	94.5	13.6	8.2	1.67
6.	5 + Ca_3PO_4 Gel Eluate	.09	695	6.5	3.8	1.7

[a] Unit = 1 μM 6,8 dimercaptoöctanoic acid, Lip (SH)$_2$, oxidized/hr. System, Table I. [b] Unit (Fraction B) = 1 μM CO_2 hr. evolved in the presence of excess fraction A. Dismutation system; 200 μM K phosphate, *p*H 6; 50 μM K pyruvate; 1 μM $MnSO_4$; 0.5 μM DPN; 0.2 μM DPT; 0.1 μM CoA; 10 μM cysteine; 2000 units lactic dehydrogenase; 10 units P-transacetylase; 10 units (Sp. Act. **340**); Fraction A. (contains lipoic acid; no rate increase on adding lipoic acid). [c] Per cent. saturation with ammonium sulfate at 0°, precipitate used. [d] Units/mg. protein.

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CoA, also occurs, *i.e.*, reaction 1. Thus over a 200-fold range of purity, the lipoic dehydrogenase activity of *E. coli* fraction B, at least in high substrate concentration, is sufficient to account for the total rate of oxidative decarboxylation of pyruvate. These data confirm the previous suggestions[4,5] that the B fraction of *E. coli* is a dehydrogenase enzyme common to the keto acid systems and that it links lipoic acid to DPN.

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MICROBIOLOGICAL TRANSFORMATIONS OF STEROIDS. IX. DEGRADATION OF C_{21} STEROIDS TO C_{19} KETONES AND TO TESTOLOLACTONE

Sir:

In continuing our studies on microbiological transformations of steroids,[1,2,3] we wish to report the degradation of C_{21} compounds, particularly progesterone (I), to C_{19} compounds: *e.g.*, 4-androstene-3,17-dione (II), 6β-hydroxy-4-androstene-3,17-dione (III) and testololactone (IV).[4] These results are given in the table.

BIOCONVERSION OF C_{21} TO C_{19} STEROIDS

Substrate	Product	Microörganism
Progesterone (I)	Androstenedione (II) + other oxygenated steroids	*Gliocladium catenulatum*, *Penicillium lilacinum* Thom, *Aspergillus flavus*
	6β-Hydroxyandrostenedione (III)	*Gliocladium catenulatum*
	Testololactone (IV) + other oxygenated steroids	*Aspergillus flavus*, *Penicillium adametzi*
17α-Hydroxyprogesterone (VI)	Testololactone (IV) + other oxygenated steroids	*Aspergillus flavus*
Other substrates	(5)	

Gliocladium catenulatum (A.T.C.C. 10523) converts I to II and III, while a strain of *Penicillium lilacinum* Thom[6] converts I to II plus other oxygenated steroids. The fermentation and extraction techniques used have been previously described.[1,2]

Turfitt[7] has shown that degradation of the side chain of 4-cholestenone to a C_{20} compound, 3-keto-4-etiocholenic acid, is accomplished by *Proactinomyces*.

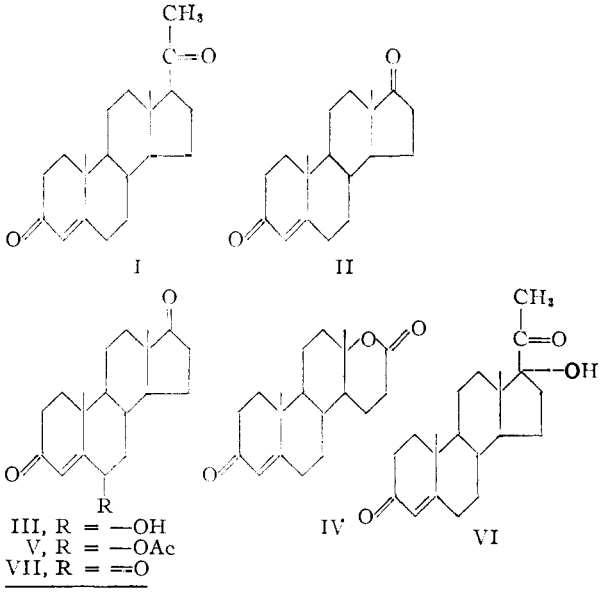
In these microbiological transformations, we have found that it is possible to degrade the side chain of C_{21} steroids to C_{19} compounds by a simple and novel one step process without the necessity of protecting the sensitive 3-keto-Δ^4 system.

The methylene chloride extractives of the beer from the *Gliocladium* were chromatographed over alumina to yield II, m.p. 174–176°, $[\alpha]^{23}D$ +194° (*c* 0.8695 in $CHCl_3$), (*Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.53; H, 8.84 and III, m.p. 190–192°, $[\alpha]^{23}D$ +107° (*c* 0.6685 in $CHCl_3$) (*Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.39; H, 8.47). Compound III yielded a monoacetate (V), m.p. 202–205°, $[\alpha]^{23}D$ +114° (*c* 0.9353 in $CHCl_3$) (*Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.97; H, 8.01). By similar techniques the beer from the *Penicillium* yielded II from I.

The physical constants of III and V are identical with those reported by Ehrenstein.[8] Oxidation of III produced 4-androstene-3,6,17-trione (VII), m.p. 220–224°, whose infrared spectrum was identical with an authentic sample.

Microbiological transformation of progesterone (I) and 17α-hydroxyprogesterone (VI) with *Aspergillus flavus* yielded testololactone (IV), m.p. 210–212°, $[\alpha]^{23}D$ +43° (*c* 1.00 in $CHCl_3$), (*Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.61; H, 8.51); androstenedione (II) and other oxygenated steroids. Similarly I was transformed to IV by *Penicillia*. The product obtained by these bioconversions was identical in all physical properties with IV obtained by chemical means,[4] the structure of which has been definitely established.[9]

When the fermentation is interrupted before the substrate is completely transformed, II as well as IV can be isolated; however, when the substrate is



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