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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RECEIVED OCTOBER 2, 1953

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.*

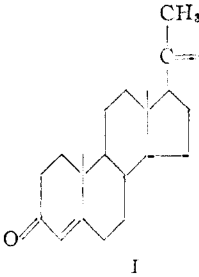
In these microbiological transformations, we have found that it is possible to degrade the side chain of C_{21} steroids to C_{19} compounds by a simple and novel one step process without the necessity of protecting the sensitive 3-keto-Δ^4 system.

The methylene chloride extractives of the beer from the *Gliocladium* were chromatographed over alumina to yield II, m.p. 174–176°, $[\alpha]^{23}\text{D}$ +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°, $[\alpha]^{23}\text{D}$ +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°, $[\alpha]^{23}\text{D}$ +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.

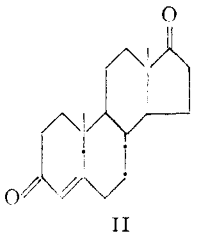
The physical constants of III and V are identical with those reported by Ehrenstein.[8] Oxidation of III produced 4-androstene-3,6,17-trione (VII), m.p. 220–224°, whose infrared spectrum was identical with an authentic sample.

Microbiological transformation of progesterone (I) and 17α-hydroxyprogesterone (VI) with *Aspergillus flavus* yielded testololactone (IV), m.p. 210–212°, $[\alpha]^{23}\text{D}$ +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]

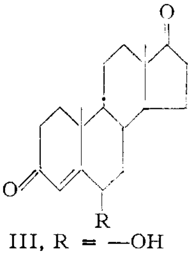
When the fermentation is interrupted before the substrate is completely transformed, II as well as IV can be isolated; however, when the substrate is



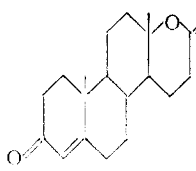
I



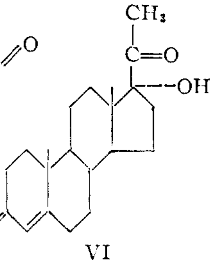
II



III, R = —OH
V, R = —OAc
VII, R = =O



IV



VI

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[1] D. H. Peterson, H. C. Murray, S. H. Eppstein, P. D. Meister, L. M. Reineke, A. Weintraub and H. M. Leigh, THIS JOURNAL, **75**, 421 (1953), and earlier references.

[2] H. C. Murray and D. H. Peterson, U. S. Patent 2,602,769, *C.A.* **46**, 8331 (1952).

[3] Microbiological transformations of steroids have also been reported by D. Perlman, *et al.*, THIS JOURNAL, **74**, 2126 (1952); J. Fried, *et al*, *ibid.*, **74**, 3962 (1952); O. Mancera, *et al.*, *ibid.*, **74**, 3711 (1952); and F. W. Kahnt, *et al*, *Experientia*, **8**, 422 (1952).

[4] H. Levy and R. P. Jacobsen, *J. Biol. Chem.*, **171**, 61 (1947).

[5] Studies have indicated that desoxycorticosterone and Reichstein's Compounds S can be converted to 4-androstene-3,17-dione (II) or testololactone (IV) and other oxygenated steroids by these cultures as well as by various *Aspergilli* and *Penicillia.*

[6] Oxidation of 14α-hydroxyprogesterone to 14α-hydroxy-4-androstene-3,17-dione by this microörganism was reported by our group at 123rd Meeting Am. Chem. Soc., Los Angeles, California, March 15–19, 1953 (Division of Biological Chemistry, Abstract 5C),

[7] G. E. Turfitt, *Biochem. J.*, **42**, 376 (1948).

[8] C. P. Balant and M. Ehrenstein, *J. Org. Chem.*, **17**, 1587 (1952).

[9] M. F. Murray, R. L. Pederson, B. A. Johnson and A. C. Ott, THIS JOURNAL, to be published.

completely utilized, II could not be found. This evidence suggests that the reaction to form IV proceeds through II.

In previous papers from these laboratories,[1,2,3,10] 6 and 11 hydroxylation and reduction of the double bond in the A ring by microörganisms have been described. The present communication describes the formation of 4-androstene-3,17-dione from C_{21} steroids. It is interesting to note that these metabolic end products from steroidal substrates are similar to those produced by higher vertebrates.[11,12,13]

Acknowledgment.—Authentic testololactone was kindly furnished us by M. F. Murray and B. A. Johnson of these laboratories. For technical assistance we are grateful to J. R. Heald, J. I. Mejeur, I. N. Pratt, H. Triemstra, H. M. Woltersom and G. Staffen.

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(10) D. R. Colingsworth, M. P. Brunner and W. J. Haines, THIS JOURNAL, **74**, 2381 (1952).

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(12) J. von Euw and T. Reichstein, *Helv. Chim. Acta*, **25**, 988 (1942); **24**, 879 (1941).

(13) H. L. Mason and W. W. Engstrom, *Physiol. Rev.*, **30**, 321 (1950).

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RECEIVED OCTOBER 19, 1953

HYDROLYTIC POLYMERIZATION OF ZIRCONIUM(IV)[1]

Sir:

Granér and Sillén[2] suggested that in the hydrolysis of Bi(III) a continuous series of particles is formed, all in equilibrium with each other and ranging in size from monomers to "infinitely" large polymers, the exact distribution depending on acidity and concentration. Recently Connick and Reas[3], in an attempt to interpret solvent extraction data on Zr(IV), advanced the same hypothesis of continuous polymerization and equilibrium between the species and postulated the existence of high molecular weight particles in acidic solutions of Zr(IV). Other workers[4,5,6] drew the conclusion that only low molecular weight polymers are formed in strongly acidic media. High molecular weight polymers, not in equilibrium with the more "normal" species, are apparently formed under considerably drastic conditions (*e.g.*, lower acidity or after boiling).[7]

Since the assumption that high molecular weight polymers are in equilibrium with low molecular weight polymers and monomers appears rather improbable and since measurement of the acidity of $ZrCl_4$ solutions[8] made it unlikely that an infinite series of polymers exists at high acidities, equilibrium ultracentrifugations of Zr(IV) were carried out in chloride and perchlorate solutions.[9] The data were recently augmented and reanalyzed by a modification of the method of Lamm[10] which was suggested to us by Professor George Scatchard, details of which will be published separately. In this computation the charge of the polymer units was considered, and estimates of this charge were obtained by ultracentrifugations under a variety of conditions. Centrifugation of 0.05 *M* Zr(IV) solutions in 1 *M* HCl–1 *M* MCl (where M was Li, Na and Cs) revealed the existence of only one principal species of Zr(IV) with an apparent degree of polymerization of 3.0 and charge $Z' < 1$ per monomer unit. At considerably higher and lower acidities (3 *M* HCl and 0.1 *M* HCl–1.9 *M* NaCl) mixtures were found with apparent degree of polymerization varying between *ca.* 2 to 2.6 (3 *M* HCl) and 4 to 5.4 (0.1 *M* HCl).

Similar low degrees of polymerization were found in ultracentrifugations in perchlorate solutions (1 *M* $HClO_4$–1 *M* $NaClO_4$). In this medium the zirconium particles appeared to carry a considerable charge and hence preliminary estimation of the degree of polymerization is somewhat more uncertain than for the chloride solutions. The most probable degree of polymerization for 0.05 and 0.12 *M* Zr(IV) solutions in this medium was 3, with an outside possibility that it may be as high as 4.5. There was no indication of an increase in degree of polymerization with concentration. These results may be compared with the (weight average) degrees of polymerization (N_w) estimated by Connick and Reas.[3] These authors report $N_w = 18$ for a considerably more dilute solution (0.03 *M* Zr(IV)–1 *M* $HClO_4$–1 *M* $LiClO_4$) and a value of N_w between 10 and 300 at a higher acidity (0.17 Zr(IV)–2*M* $HClO_4$).

The results of the ultracentrifugation experiments described here thus indicate that Zr(IV) in strongly acidic solutions ($M\ H^+ > 0.1$) does not show continuous polymerization with high molecular weight products but rather forms only low molecular weight polymers with trimers apparently predominating at acidities near 1 *M*.

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(1) This document is based on work performed for the Atomic Energy Commission at the Oak Ridge National Laboratory: "Hydrolytic Behavior of Metal Ions. II," previous paper, THIS JOURNAL, **72**, 3901 (1950).

(2) F. Granér and L. G. Sillén, *Acta Chem. Scand.*, **1**, 631 (1947).

(3) R. E. Connick and W. H. Reas, THIS JOURNAL, **73**, 1171 (1951).

(4) M. Adolf and W. Pauli, *Kolloid Z.*, **29**, 173 (1921).

(5) G. Jander and K. F. Jahr, *Kolloid-Beih.*, **43**, 295 (1936).

(6) B. A. Lister and L. A. McDonald, *J. Chem. Soc.*, 4315 (1952).

(7) See, *e.g.*, R. Ruer, *Z. anorg. u. allgem. Chem.*, **43**, 282 (1905).

(8) K. A. Kraus and S. Y. Tyree, Jr., Report ORNL-499 (Sept. 1949).

(9) J. S. Johnson and K. A. Kraus, Reports ORNL-607 (1949), ORNL-1053 (1951).

(10) O. Lamm, *Arkiv Kemi, Mineral. Geol.*, **17A**, No. 25 (1944).

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RECEIVED SEPTEMBER 22, 1953

DPNH-CYTOCHROME REDUCTASE, A FERRO-FLAVOPROTEIN[1]

Sir:

Within the past year several flavoprotein enzymes have been shown to contain heavy metals as part of their prosthetic groups. Copper was identified as a constituent of butyryl CoA dehydrogen-

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(1) Paper IV in a series entitled Studies on Diphosphopyridine Nucleotide-Cytochrome c Reductase. For paper III see L. P. Vernon, H. R. Mahler and N. K. Sarkar, *J. Biol. Chem.*, **199**, 598 (1952).