oxide[10,11] III yielded $\Delta^{6,8,22}$-ergostatrien-3β,14-diol-11-one 3-acetate (IV), m.p. 188.8–192.4°; $[\alpha]_D$ +34° ($CHCl_3$); $\lambda_{max.}$ 308 mu (log ϵ = 3.84) (ether); found: C, 76.72; H, 9.65. Acid-catalyzed dehydration, followed by reacetylation led to $\Delta^{6,8,14,22}$-ergostatetraen-3β-ol-11-one acetate (V), m.p. 145.0–146.8°; $[\alpha]_D$ −82° ($CHCl_3$); $\lambda_{max.}$ 326 mu (log ϵ = 3.95), $\lambda_{max.}$ 233 mu (log ϵ = 4.18) (ether); found: C, 79.72; H, 9.54. The position of the carbonyl group at C-11 in V was established by hydrogenation over palladium-charcoal or W-7 Raney nickel[12] to form Δ^8-ergosten-3β-ol-11-one acetate (VII), m.p. 137.8–138.6°; $[\alpha]_D$ +125° ($CHCl_3$); $\lambda_{max.}$ 248 mu (log ϵ = 3.90) (ether); found: C, 78.91; H, 10.74; melting point undepressed on admixture with an authentic sample prepared by hydrogenation of VI obtained by an independent route.[2b,4]

Intermediates retaining the unsaturated side chain and suitable for conversion to cortisone were prepared by selective hydrogenation of V over W-2 nickel[13] to form $\Delta^{8,14,22}$-ergostatrien-3β-ol-11-one acetate, m.p. 127.0–128.2°; $[\alpha]_D$ +20° ($CHCl_3$); $\lambda_{max.}$ 291 mu (log ϵ = 4.06) (ether); found: C, 79.51; H, 9.92; or over W-7 nickel to form the known cortisone intermediate, $\Delta^{8,22}$-ergostadien-3β-ol-11-one acetate (VI), m.p. 131.4–131.8°; $[\alpha]_D$ +110° ($CHCl_3$); $\lambda_{max.}$ 248 mu (log ϵ = 3.95) (ether); melting point undepressed on admixture with an authentic sample.[2b,4]

Details of this work and alternate conversions of III and IV will be the subject of later communications from this Laboratory.

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THE ENZYMATIC OXIDATION OF *d*- AND *l*-β-HYDROXYBUTYRATE[1]

Sirs:

Although the *l*-isomer of β-hydroxybutyric acid is often regarded as the "naturally occurring" isomer[2] the *d*-isomer is also known to undergo biological oxidation.[3,4,5]

We have found that the mechanisms of enzymatic oxidation of the two pure isomers[6] in clear, dialyzed extracts of acetone-dried rat liver mitochondria are quite different. Such extracts contain the already known[2] diphosphopyridine nucleotide (DPN)-linked, *l*-specific β-hydroxybutyric dehydrogenase catalyzing the following reaction

$$\text{1-}\beta\text{-Hydroxybutyrate} + \text{DPN} \rightleftharpoons \text{acetoacetate} + \text{DPNH} \quad (1)$$

This reaction requires no components beyond extract, DPN and *l*-β-hydroxybutyrate. Under these circumstances *d*-β-hydroxybutyrate is not oxidized.

However such extracts will cause the reduction of DPN by the *d*-isomer if they are supplemented with adenosine triphosphate (ATP), Coenzyme A, and Mg^{++}. Such additions have no stimulatory effect on the reduction of DPN by *l*-β-hydroxybutyrate. Furthermore, the *l*-specific β-hydroxybutyric dehydrogenase is not involved in the oxidation of the d-isomer in the presence of these additional cofactors, since fractionation of the extracts yielded preparations with high activity toward *d*-β-hydroxybutyrate and little or none toward the *l*-isomer (Table I). These findings therefore suggested

TABLE I

ENZYMATIC OXIDATION OF *d*-β-HYDROXYBUTYRATE

The test system contained 0.10 ml. of dialyzed fraction of acetone-dried rat liver mitochondria, 10 μM. cysteine, 2.5 μM. ATP, 5 μM. $MgCl_2$, 50 μM. tris-(hydroxymethyl)-aminomethane buffer *p*H 8.0, 0.5 μM. CoA, 100 μM. KCl, 1.0 μM. DPN, 25 μM. *d*- or *l*-β-hydroxybutyrate and H_2O to make 1.00 ml.; temp. 20°; time, 20 min. Appearance of DPNH measured spectrophotometrically at 340 mμ.

System	Substrate	Δ log I_0/I
Complete	None	0.015
Complete	*d*-Isomer	.670
ATP omitted	*d*-Isomer	.005
CoA omitted	*d*-Isomer	.062
Mg^{++} omitted	*d*-Isomer	.149
Complete	*l*-Isomer	.040
ATP + CoA + Mg^{++} omitted	*l*-Isomer	.052

that the reduction of DPN by the *d*-isomer proceeds as follows

$$\text{d-}\beta\text{-Hydroxybutyrate} + \text{CoA} \xrightarrow{\text{ATP}} \text{d-}\beta\text{-hydroxybutyryl-CoA} \quad (2)$$

$$\text{d-}\beta\text{-Hydroxybutyryl-CoA} + \text{DPN} \rightleftharpoons \text{acetoacetyl-CoA} + \text{DPNH} \quad (3)$$

Further evidence for this formulation follows: In the absence of DPN but with hydroxylamine present as a "trapping" agent the *d*-isomer forms a hydroxamic acid derivative, detected by colorimetry and paper chromatography.[7] This reaction requires the presence of ATP, Mg^{++} and CoA. The formation of acetoacetyl-CoA as the end-product of the over-all reaction was established by the finding that citrate was formed as product of oxidation of *d*-β-hydroxybutyrate when oxalacetate, excess CoA, and "condensing enzyme" were present, *via* the following known reactions[8]

$$\text{Acetoacetyl-CoA} + \text{CoA} \rightleftharpoons 2\ \text{acetyl-CoA} \quad (4)$$

$$\text{Acetyl-CoA} + \text{oxalacetate} \rightleftharpoons \text{citrate} + \text{CoA} \quad (5)$$

Free acetoacetate formed no citrate under these conditions.

(1) This work was supported in part by grants from the Nutrition Foundation, Inc., the U. S. Public Health Service, and the John Simon Guggenheim Foundation.

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Since *l*-β-hydroxybutyrate also forms a hydroxamic acid in the presence of ATP, CoA, Mg^{++} and hydroxylamine (*cf.* equation (2)) but causes little if any reduction of DPN (Table I) it would appear that reaction (3) is responsible for the optical specificity of the over-all scheme.

The dehydrogenase catalyzing reaction (3), which has been found to be reversible, may be identical with that recently described by Lynen, *et al.*[9] In view of the present work it would appear probable that the β-hydroxybutyryl-CoA participating in this reaction contains the *d*-isomer. Since reactions (3) and (4) are reversible, *d*-β-hydroxybutyrate may be regarded as a "naturally occurring" metabolite in the form of its CoA derivative and may possibly be an intermediate in the enzymatic oxidation and synthesis of butyric acid.

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THE ROLE OF N^{15} GLYCINE, GLUTAMINE, ASPARTATE AND GLUTAMATE IN HYPOXANTHINE SYNTHESIS[1]

Sir:

Previous investigations *in vivo*[2,3,4] have shown that carbon dioxide, formate and glycine are precursors of the carbon atoms of the purine ring, and that glycine also supplies one of the four nitrogen atoms of the ring, nitrogen atom 7. It has also been shown that in an extract of pigeon liver where many of the side reactions complicating quantitative work *in vivo* are negligible, carbon dioxide, formate and glycine combine in the definite molecular proportion of 1:2:1 in the synthesis of hypoxanthine.[5] With the use of this technique of comparing the number of molecules of two different substrates utilized in the synthesis of hypoxanthine by pigeon liver extract, a search has been made for the nitrogen precursors of the three nitrogen atoms of the purine ring not supplied by glycine.[5,6] The N^{15}-labeled substrates were individually incubated with pigeon liver extract synthesizing hypoxanthine from glycine-1-C^{14}. At the termination of the incubation the hypoxanthine formed was converted to uric acid by the action of xanthine oxidase. After the addition of carrier, the uric acid was isolated, purified and analyzed for C^{14} and N^{15} content. The C^{14} of the radioactive glycine and the N^{15} of the other labeled substrates were determined and found to have remained sufficiently constant during the 30-minute incubation. From these analytical values it was possible to calculate the number of atoms of N^{15} which each of the nitrogenous compounds tested contributed to the synthesis of each molecule of hypoxanthine formed *de novo* from radioactive glycine. These ratios are reported in the accompanying table. It may be seen that, although ammonia is readily incorporated into purines *in vivo*, its low incorporation here indicates that it is not an immediate precursor of any one of the hypoxanthine nitrogen atoms. The other nitrogenous substrates, however, contributed significantly to hypoxanthine synthesis. The amide group of glutamine supplied approximately two atoms of nitrogen, and the α-amino group of the other substrates, N^{15} labeled glycine, aspartic and glutamic acids supplied one atom each. These integer ratios suggested that these substrates were contributing to specific nitrogen atoms of the purine ring. Degradation of the uric acid samples was carried out into fractions containing nitrogen atoms 1 and 3 combined, 7 and 9 combined and 7 alone. The analyses from the several experiments revealed considerable variation in the pattern of distribution of the N^{15} in the purine molecule and supported the belief that the substrates were specific donors. In the glycine N^{15} experiment the isotope was primarily in position 7; in the glutamine experiment the N^{15} donated by the amide group was found to be about half in the 9 atom and half in the 1 + 3 fraction. In the aspartic and glutamic acid experiments, almost all of the N^{15} was found in the 1 + 3 fraction, with a small amount in the 7 + 9 fraction. In the 7 + 9 fraction, therefore, the 7 atom is supplied by the known precursor glycine, and the 9 atom by the amide group of one of the two molecules of glutamine used in hypoxanthine synthesis. The 1 + 3 fraction has not been further separated, but the biological similarity of the transaminating dicarboxylic acids favors the probability that their α-amino groups both supply the same one atom of this pair, and that the other nitrogen atom of the pair is donated by the amide group of

TABLE I

LOCATION OF N^{15} IN THE PURINE RING

Expt.	N^{15} labeled substrate	Moles of N^{15} utilized for hypoxanthine synthesis per mole of C^{14} labeled glycine	N^{15} in various N atoms[a] of purine molecule (atom per cent. excess N^{15}) 1 + 3	7 + 9	7	9
1	NH_4Cl	0.27	...	...	...	...
2	Aspartic	1.20	0.091	0.009	...	...
3	Glutamic	1.20	.185	.025	...	...
4	Glutamine (amide N^{15})	1.90	.186	.176	.018	0.334[b]
5	Glycine	1.00	.058	.378	.750	.058

[a] The distribution of N^{15} in the purine ring in individual experiments was determined after further dilution of the original sample with varying amounts of uric acid. Therefore, only the N^{15} values of the nitrogen fraction of uric acid within each experiment are comparable. [b] Estimated from the N^{15} determination of nitrogen atom 7 and nitrogen fraction 7 + 9.

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