Unlike rabbit γ-globulin, normal human γ-globulin (Fraction II or γ_2) contains approximately one mole each of N-terminal aspartic and glutamic acids per 160,000 g. It also yields 0.1 to 0.2 mole of N-terminal serine and small amounts of an unidentified DNP-derivative. In contrast, the three myeloma globulins of mobility -1.1 *u* had two moles of N-terminal aspartic acid per 160,000 g., and in accord with their greater physical homogeneity, were entirely free or nearly so of other N-terminal groups. The myeloma globulin of mobility -0.7 *u* was essentially devoid of N-terminal glutamic or aspartic acids, whereas these occurred in an almost equimolar ratio in the heterogeneous β-globulin.

This appears to be the first demonstration of a difference from normal in the chemical structure of serum proteins elaborated in any disease. It may, however, be interpreted in terms of the physiological occurrence of three (or more) gamma globulins only one of which is synthesized profusely by a given patient with multiple myeloma. Of these proteins one may contain at least two peptide chains both terminating in glutamic acid, another two chains with only aspartic acid as the amino end-group, and the third has neither amino acid in the N-terminal position. This hypothesis is supported by results communicated to us by McFadden and Smith[4] and confirmed in this laboratory, in which it was found that human γ-globulin subfraction II-1,2 has 1.7 times as many moles of N-terminal glutamic acid as of aspartic, whereas subfraction II-3 has one mole of each. There is no correlation of N-terminal groups with the isoelectric point, for in three proteins with a *p*I of 7.3 to 7.5, the molar ratio of N-terminal glutamic to aspartic ranged over twenty-fold. Since physicochemical analysis has indicated great variation in the nature of myeloma globulins,[6] further terminal group analysis may reveal new end-groups and other information of interest to the study of normal serum proteins and of antibody globulins.[9]

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RECEIVED MAY 6, 1953

REVERSIBLE CATALYTIC CLEAVAGE OF HYDROXY-AMINO ACIDS BY PYRIDOXAL AND METAL SALTS

Sir:

Pyridoxal and salts of copper, iron and aluminum catalyze the deamination of serine to pyruvate.[1] We have now found that while threonine and allothreonine undergo similar reactions they are much more rapidly cleaved to glycine and acetaldehyde throughout the *p*H range 3–12 in the presence of pyridoxal and copper, iron or aluminum salts. The reactions are readily reversible. Some analyses on reaction mixtures heated at 100° are given in Tables I and II. Similar results were obtained by allowing the reaction to proceed at room temperature for a few days. The amino acid concentrations were determined microbiologically or by the ninhydrin color reaction after separation on a column of cation exchange resin.

The cleavage of serine to glycine and formaldehyde can be demonstrated readily at *p*H 7 where its deamination is slow as can the conversion of formaldehyde and glycine to serine and pyruvate.

Other aldehydes such as propionaldehyde, glyoxylic acid, and pyridoxal can be condensed with glycine under the same conditions to yield a variety of β-hydroxy amino acids.

The presence in animal livers of an enzyme which splits threonine, allothreonine, β-hydroxyvaline, β-hydroxynorvaline and β-phenylserine to glycine and carbonyl compounds has been reported.[2] The reactions are inhibited by carbonyl reagents and despite the lack of decrease in activity in livers of vitamin B_6-deficient rats we predict that this will be found to be a pyridoxal-containing enzyme. A similar enzyme which splits serine is stated to require a cofactor related to folic acid and not to be inhibited by carbonyl reagents. However, vitamin B_6 is also required by both bacteria[3] and chicks[4] for synthesis of serine from glycine.

The combination of the following three vitamin B_6-catalyzed reactions provides an attractive mechanism of amino acid biosynthesis.

TABLE I

CLEAVAGE OF THREONINE TO GLYCINE AND ACETALDEHYDE

Reaction mixtures 20 mM. in threonine, *p*H 5, were heated 30 min. at 100°.

Additions, millimoles per l.		Products, millimoles per l.			
Pyridoxal	Alum[a]	Threonine	Glycine	Acetaldehyde	Ammonia
0	2	19.2	0.0		
10	0	17.5	1.2		
10	2	3.7[b]	12.5	14.0	1.3

[a] $KAl(SO_4)_2 \cdot 12H_2O$. [b] A small amount of allothreonine was also produced.

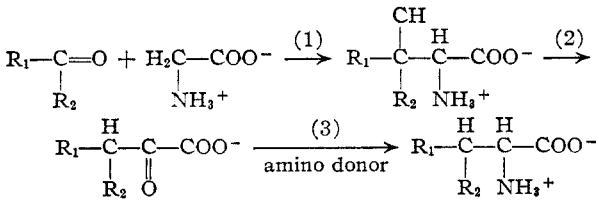
TABLE II

THE REVERSIBILITY OF THREONINE CLEAVAGE

Reaction mixtures containing 10 mM. pyridoxal and 2 mM. alum at *p*H 5 were heated at 100°.

Reactants, millimoles per l.			Heating time, hr.	Threonine, found millimoles per l.
Threonine	Glycine	Acetaldehyde		
20	20	80	0.25	11.7
20	20	80	2.0	4.4
0	40	100	0.25	2.6[a]
0	40	100	2.0	3.7[a]

[a] Allothreonine was also produced in similar amounts.



The non-enzymatic reactions catalyzed by pyridoxal and metal salts have been carried out for

(1) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).

(2) G. Ya. Vilenkina, *Doklady Akad. Nauk S.S.S.R.*, **84**, 559 (1952), from *C. A.*, **46**, 10227 (1952), and preceding papers.

(3) J. Lascelles and D. D. Woods, *Nature*, **166**, 649 (1950).

(4) S. Deodhar and W. Sakami, *Fed. Proc.*, **12**, 195 (1953).

each of the steps leading to the formation of aminobutyric acid from acetaldehyde and animal livers appears to contain enzymes which can catalyze these same reactions. The enzyme reported by Vilenkina[2] should catalyze reactions (1) and Lien and Greenberg[5] have reported conversion of threonine to aminobutyric acid, apparently by reactions (2) and (3) in rat livers. Though this synthetic pathway may not be used by animals it may be of importance in some organisms.

(5) O. G. Lien, Jr., and D. M. Greenberg, *J. Biol. Chem.*, **200**, 367 (1953).

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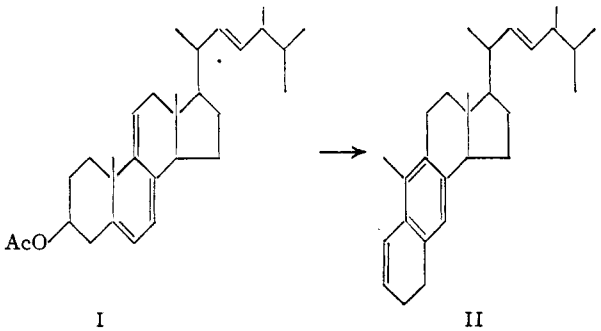
THE REARRANGEMENT OF DEHYDROERGOSTERYL ACETATE TO A *s*-OCTAHYDROANTHRACENE DERIVATIVE

Sir:

On treatment of a chloroform solution of dehydroergosteryl acetate (I) with catalytic amounts of hydrogen chloride at room temperature a skeletal rearrangement of the steroid takes place. The pure product (II) obtained in a yield of about 30% lacks an oxygen function and shows an ultraviolet absorption spectrum characteristic of an aromatic ring with one conjugated double bond, $\lambda_{max.}$ (isooctane) 222, 227, 266, 296, and 308 mμ. (ε 26,100, 27,100, 18,600, 2,760, 2,220, respectively); $\lambda_{max.}$ (CS_2) 968 cm.$^{-1}$; m.p. 105–107°; $[\alpha]^{20}D$ −70° ($CHCl_3$); *Anal.* Calcd. for $C_{28}H_{40}$: C, 89.29; H, 10.70. Found: C, 88.96; H, 10.74. It is proposed that, by the rupture of the C_1–C_{10} bond and reattachment of C_1 to C_6, 1,2,3,4,7,8-hexahydro-3′-(5,6-dimethyl-3-heptenyl-2)-2,10-dimethyl-1,2-cyclopentanthracene (II) is formed. (Positions 7,8 and 3,4 for the conjugated double bond have not been ruled out experimentally.) Kinetic measurements by ultraviolet spectrophotometry show that this rearrangement is first order in steroid and approximately second order (1.85) in hydrogen chloride. The reaction rate constant is equal to 0.146 ± 0.003 liter^2 moles^{-2} sec.$^{-1}$ at 20°.



I II

By catalytic hydrogenation (PtO_2, ethyl acetate–acetic acid) the double bond in the side chain and the conjugated olefinic double bond are saturated to give the corresponding *s*-octahydroanthracene derivative (III), m.p. 106–107°; $[\alpha]^{20}D$ +21° ($CHCl_3$); $\lambda_{max.}$ (isoöctane) 273, 278 and 282 mμ (ε 670, 550 and 695 respectively), $\lambda_{min.}$ 247 mμ (ε 95); *Anal.* Calcd. for $C_{28}H_{44}$: C, 88.34; H, 11.65. Found: C, 88.42; H, 11.47. Oxidation of II with 70% nitric acid and subsequent esterification of the resulting compound with diazomethane leads to 1-methyl-2,3,5,6-tetracarbomethoxybenzene (IV), m.p. 121–123°; *Anal.* Calcd. for $C_{15}H_{16}O_8$: C, 55.55; H, 4.97. Found: C, 55.43; H, 5.06. The structure of IV was confirmed by its comparison with a sample obtained by an analogous oxidation of 9-methyl-*s*-octahydroanthracene. Compound IV, incidentally, was found to be identical with the methyl tetracarbomethoxybenzene obtainable by the nitric acid oxidation of various steroids.[1] From the analogous oxidation of 9-methyl-*s*-octahydrophenanthrene we obtained pentacarbomethoxybenzene instead of the expected, unknown 1-methyl-2,3,4,5-tetracarbomethoxybenzene (V).

We are considering the possibility that this type of facile rearrangement, *i.e.*, the transformation of steroids into anthracene derivatives, is involved in spontaneous carcinogenesis.

(1) (a) H. H. Inhoffen, *Ann.*, **494**, 122 (1932); (b) A. Windaus and G. Zühlsdorff, *ibid.*, **536**, 204 (1938); (c) M. Müller, *Z. physiol. Chem.*, **233**, 223 (1935).

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RECEIVED APRIL 30, 1953

ENZYMES OF THE FATTY ACID CYCLE. II. ETHYLENE REDUCTASE[1]

Sirs:

We have recently reported on the identification and isolation of β-keto thiolase and β-keto reductase.[2] Similar results have been obtained in other laboratories.[3,4,5] Through the combined action of these two enzymes the cell elongates the chain of the CoA thioester derivatives of fatty acids by the addition of a C_2 carbon chain from acetyl-S-CoA forming the corresponding β-hydroxy-CoA-thioester derivatives. In this way β-hydroxy-butyryl-S-CoA is formed from acetyl-S-CoA (Reactions 1 and 2).

$$2CH_3\text{—}CO\text{—}S\text{—}CoA \rightleftharpoons CH_3\text{—}CO\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA + HS\text{—}CoA \quad (1)$$

$$CH_3\text{—}CO\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA + DPNH + H^+ \rightleftharpoons CH_3\text{—}CHOH\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA + DPN^+ \quad (2)$$

$$CH_3\text{—}CHOH\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA \rightleftharpoons CH_3\text{—}CH{=}CH\text{—}CO\text{—}S\text{—}CoA + H_2O \quad (3)$$

$$\text{Leuco-safranine} + CH_3\text{—}CH{=}CH\text{—}CO\text{—}S\text{—}CoA \longrightarrow \text{Safranine} + CH_3\text{—}CH_2\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA \quad (4)$$

(1) This work was supported in part by a grant from the Research Foundation of Germany. The following abbreviations are used: Coenzyme A, CoA-SH; acyl coenzyme A derivatives, acyl-S-CoA; oxidized and reduced diphosphopyridine nucleotide, DPN$^+$ and DPNH; reduced triphosphopyridine nucleotide, TPNH; flavinadenine dinucleotide, FAD; micromoles, μM.

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

(3) J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

(4) A. L. Lehninger and G. D. Greville, *ibid.*, **75**, 1515 (1953).

(5) D. E. Green and S. Mii, *Federation Proc.*, **12**, 211 (1953).