TABLE I

DISTRIBUTION OF N^{15} IN HISTIDINE

Experiment No. →	1		2		3[a]	
	N^{15} atom % excess[b]	N derived from amide N, %	N^{15} atom % excess	N derived from amide N, %	N^{15} atom % excess	N derived from amide N, %
Histidine	2.10	6.5 (8.1)[c]	2.68	8.2 (10.3)	2.80	8.6 (10.7)
α-NH_2-N	.71	2.2 (2.8)	.89	2.7 (3.4)	1.00	3.1 (3.8)
N-3	.55	1.7 (2.1)	.82	2.5 (3.1)	.57	1.8 (2.2)
N-1 calcd.[d]	5.0	15 (19)	6.3	19 (24)	6.8	21 (26)
N-1 found	5.0	15 (19)				
Protein glutamic acid N	.73	2.2 (2.8)				

[a] Procedure was identical to experiments 1 and 2 except that asparagine replaced ammonia in the medium. [b] All samples were diluted four-fold with unlabeled N prior to analysis except for the glutamic acid isolated from the enzyme digest which was diluted 32-fold. [c] The values in the brackets are the per cent. amide N incorporated corrected for dilution by the original inoculum (approximately 20% of the final 500 mg. of protein). [d] Calculated by difference $3 \times 2.1 - 0.71 - 0.55$.

This culture (250 ml.) was used to inoculate one liter of the same medium, at which time 52 mg. of isotopic glutamine (5 mg. amide-N 32.5 atom % excess N^{15}) was added, an addition which was repeated after one hour. After a total of 3.5 hours of incubation at 37° with shaking, the cells were separated by centrifugation and the protein was obtained by treatment with cold and hot trichloroacetic acid, ethanol, ethanol–chloroform–ether mixture, and ether. Histidine was precipitated and recrystallized as the bis-3,4-dichlorobenzene sulfonate,[9] after prior isolation from the hydrolyzed protein as the mercury salt.[10] Stepwise degradation to urocanic acid and glutamic acid was effected by successive digestion with heat-treated and unheated extracts of *Pseudomonas fluorescens*.[11] By the first digestion ammonia corresponding to the α-amino group of histidine was obtained, the second digestion liberating nitrogen 3 of the imidazole ring as ammonia. Glutamic acid (N-1 of histidine) was recovered from the enzyme digests by the Foreman procedure[12] and recrystallized as the hydrochloride. Determinations of the N^{15} concentration in the different samples were carried out with a Process and Instruments mass spectrometer, which was kindly made available to us by Dr. S. Graff.

The high incorporation of glutamine amide nitrogen into N-1 in the presence of a 180-fold excess of ammonia (experiments 1 and 2) or a 90-fold excess of asparagine amide-N (experiment 3) points to the participation of glutamine or a compound derived from it in an early stage of histidine biosynthesis. Since our data (N^{15} incorporation into the α-amino group of histidine and protein glutamic acid) showed that glutamine is concentrated by the cells in preference to ammonia, the possibility might be visualized that N-1 is derived from intracellular ammonia originating in the amide group and not in equilibrium with the α-amino group of glutamic acid. The results obtained (experiment 3) with cells grown on asparagine as the major nitrogen source argue against this possibility.

The question arises whether the amide group of glutamine participates directly in histidine synthesis by primary formation of an amino sugar or an amino aldehyde or indirectly by group transfer from an intermediate such as guanine.

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(9) H. B. Vickery, *J. Biol. Chem.*, **143**, 79 (1942).

(10) G. L. Foster and D. Shemin, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., p. 330.

(11) H. Tabor and A. Hayaishi, *J. Biol. Chem.*, **194**, 171 (1952); A. H. Mehler and H. Tabor, *ibid.*, **201**, 775 (1953).

(12) D. B. Jones and O. Moeller, *ibid.*, **51**, 103 (1922).

RELATIVE RATE CONSTANTS FOR REACTION OF OH RADICAL WITH SULFURIC ACID, FORMIC ACID AND CEROUS ION

Sir:

Hydrogen does not readily react[1,2] with OH radical in 0.4*M* sulfuric acid. Allen[3] has suggested that this may be due to complexes, such as $H_2SO_5^-$ or HSO_4, formed by OH radical with sulfuric acid. The relative reactivity of OH radical with sulfuric acid has been quantitatively determined by a study of the gamma irradiation of 0.4*M* sulfuric acid solutions containing mixtures of ceric ion, cerous ion and formic acid.

The 100 e.v. yields of the initial products H, OH, H_2 and H_2O_2 in the radiolysis of water are denoted by G_H, G_{OH}, G_{H_2} and $G_{H_2O_2}$. The 100 e.v. yield of any product in the radiolysis of aqueous solutions is denoted by G(product). In the radiolysis of ceric ion–cerous ion–formic acid mixtures, $G(Ce^{++++})$ increases with decreasing $(Ce^{+++})/(HCOOH)$ ratio at any constant cerous ion concentration while at constant $(Ce^{+++})/(HCOOH)$ ratio $G(Ce^{++++})$ decreases with decreasing total concentration of cerous ion and formic acid. These data are quantitatively interpreted by the assumption that three solutes compete with each other for reaction with OH radical

$$Ce^{++} + OH \longrightarrow Ce^{+++} + OH^- \quad (1)$$

$$HCOOH + OH \longrightarrow HCOO + H_2O \quad (2)$$

$$H_2SO_4 + OH \longrightarrow HSO_4 + H_2O \quad (3)$$

Whether it is H_2SO_4, HSO_4^- or $SO_4^=$ which reacts with OH radical has not been determined but it will be assumed for kinetic treatment to be H_2SO_4 in

(1) A. O. Allen, C. J. Hochanadel, J. A. Ghormley and T. W. Davis, *J. Phys. Chem.*, **56**, 575 (1952).

(2) T. J. Sworski, *J. Chem. Phys.*, **21**, 375 (1953).

(3) A. O. Allen, *Radiation Research*, **1**, 85 (1954).

0.4M concentration. In these mixtures, ceric ion is reduced by H, HO_2, HCOO and H_2O_2 while cerous ion is oxidized by OH and HSO_4.

The experimental data are fairly well represented by the equation

$$G(Ce^{+++}) = 2G_{H_2O_2} + G_H - G_{OH} + 2G_{OH} \Big/ \left[1 + \frac{k_1(Ce^{+++}) + k_3(H_2SO_4)}{k_2(HCOOH)}\right]$$

It is assumed in this equation that (a) HSO_4 radical oxidizes cerous ion but does not react with formic acid and (b) HCOO radical reduces[4,5] ceric ion but does not oxidize cerous ion. The previously reported[6] values of G_H = 3.70, G_{OH} = 2.92, G_{H_2} = 0.39 and $G_{H_2O_2}$ = 0.78 are used with a correction made for the decrease in $G_{H_2O_2}$ (with a concomitant increase in G_{OH}) by cerious ion[7] and formic acid. The equation fairly well represents the data with values for k_1/k_2 of 1.70, k_2/k_3 of 380 and k_1/k_3 of 650.

The decreased reactivity of hydrogen with OH radical in 0.4M sulfuric acid must then be attributed to competition of sulfuric acid with hydrogen for reaction with OH radical. The occurrence of reaction 3 in sulfuric acid solutions must of necessity be considered in those cases where the reactions of OH radical differ from those of the radical formed in reaction 3.

(4) T. J. Sworski, THIS JOURNAL, **77**, 1074 (1955).
(5) H. E. Spencer and G. K. Rollefson, *ibid.*, **77**, 1938 (1955).
(6) T. J. Sworski, *ibid.*, **76**, 4687 (1954).
(7) T. J. Sworski, *Radiation Research*, in press.

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2α-HYDROXYLATION OF CORTISOL IN THE GUINEA PIG[1]

Sir:

Recently the isolation of 6β-hydroxycortisol and the partial characterization of two other $C_{21}O_6$ urinary metabolites of cortisol (I) (hydrocortisone) in the guinea pig has been described.[2] 6β-Hydroxycortisol and one of the partially characterized steroids, termed[2] steroid IIa, have also been isolated from the urine of untreated guinea pigs[3] and in markedly elevated concentrations from the urine of guinea pigs with leukemia and liposarcoma.[4] The purpose of this communication is to report on the identification of steroid IIa as 2α-hydroxycortisol (II). The identification was achieved by elemental analysis of the diacetate, by spectroscopic evidence in alkali and by comparison with synthetic II obtained as the major $C_{21}O_6$ product from the reaction of I-21-acetate with lead tetraacetate. This finding represents the first instance of 2α-hydroxylation in a mammal. The only steroids with a hydroxyl at C-2 hitherto found in nature have been known to occur among the sapogenins from plant origin.

Steroid IIa diacetate was isolated, as previously described,[2] from a pool of guinea pig urine. After five crystallizations from methanol an analytical sample was obtained, m.p. 224–230°, $\lambda_{max.}^{methanol}$ 242 mμ (16,000). *Anal.* Calcd. for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41; Found: C, 64.91; H, 7.60. The infrared spectrum and the spectrum in sulfuric acid have been reported previously.[2] The diacetate was hydrolyzed with $KHCO_3$ under the conditions described by Sondheimer, *et al.*[5] Chromatography of the reaction mixture on paper in the chloroform–formamide system gave the free steroid IIa which was identical (running rate on paper, infrared spectrum and spectrum in sulfuric acid) with steroid IIa isolated directly from guinea pig urine. The latter after extensive chromatographic separation and crystallization from ethyl acetate–benzene, exhibited m.p. 185–190°, ν^{KBr} −3300 (hydroxyl), 1704 (C-20 carbonyl), 1669 (C-3 carbonyl), 1616 (Δ⁴-double bond) cm.$^{-1}$. The material showed a green fluoresence in sulfuric acid and the spectrum in sulfuric acid immediately after dissolving in acid had the following bands: 500 (0.28), 383 (0.19), 292 (0.60) and 239 (0.47) mμ; two hours after dissolving in acid: 485 (0.22), 390 (0.19), 330 (0.37) (shoulder), 289 (0.49) and 240 (0.55) mμ. (Values in parentheses are the optical densities for *ca.* 50γ of material in 3 cc.). Steroid IIa in tetramethylammonium hydroxide (0.066 N) showed the following *uniquely characteristic* spectrum of a 2-hydroxy-Δ⁴-3-keto steroid described by Meyer[6]: 2–3 minutes in alkali, λmax. 242 mμ (ε = 14,000); 30 minutes at 60°, λmax. 231 mμ (22,600), λinflection 252–256 mμ (7,560), λminimum 290 mμ (920), λmax. 355 mμ (2,300); after acidification, λmax. 259, λinflection 290 mμ.

Synthetic II was prepared by treating I-acetate with lead tetraacetate according to Sondheimer, *et al.*[5] Since no crystalline 2-acetoxycortisol could be obtained by chromatography,[7] the reaction mixture was hydrolyzed with $KHCO_3$[5] and chromatographed twice on paper. Crystalline 2α-hydroxycortisol, m.p. 188–192°, was isolated as the major $C_{21}O_6$ reaction product which was identical (infrared, spectrum in sulfuric acid, spectrum in alkali and mobility on paper) with steroid IIa. The structure 2α-hydroxycortisol was assigned to the synthetic material because of its method of preparation which is known to lead to 2-acetoxysteroids.[5,8,9] In addition, II is different from the previously described more polar 6β-hydroxycortisol,[2] the other possible product of the reaction at an allylic position. (The 6α-hydroxy structure can be excluded owing to the fact that it would be of slower mobility and exhibit an entirely different spectrum in alkali[6].) The 2α-hydroxy configuration follows from the fact that hydrolysis with

(1) The work was supported in part by Research Grant No. NSF-G664, National Science Foundation.
(2) S. Burstein and R. I. Dorfman, *J. Biol. Chem.*, **213**, 581 (1955).
(3) S. Burstein, R. I. Dorfman and E. M. Nadel, *ibid.*, **213**, 597 (1955).
(4) E. M. Nadel, S. Burstein and R. I. Dorfman, *Proc. Amer. Assoc. Cancer Res.*, **2**, 37 (1955).
(5) F. Sondheimer, St. Kaufman, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).
(6) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).
(7) S. Burstein and R. I. Dorfman, THIS JOURNAL, **77**, 4668 (1955).
(8) E. Seebeck and T. Reichstein, *Helv. Chim. Acta.*, **27**, 948 (1944).
(9) G. Rosenkranz, O. Mancera and F. Sondheimer, THIS JOURNAL, **77**, 145 (1955).