

UTILIZATION OF PTEROYLGLUTAMIC ACID  
CONJUGATES IN THE *IN VITRO* SYNTHESIS OF  
*L. CITROVORUM* ACTIVITY<sup>1</sup>

Sir:

The demonstration that liver slices convert synthetic pteroylglutamic acid (I) to a substance(s) possessing microbiological activity for *L. citrovorum*<sup>2</sup> led us to study the ability of chick liver tissue to convert pteroyldiglutamic acid (II) and pteroyltriglutamic acid (III) to a substance(s) possessing *L. citrovorum* activity. Using the procedure of Nichol and Welch<sup>2</sup> and employing the modifications reported previously<sup>3</sup> it was observed (Table I) that liver slices from chicks deficient in I synthesized *L. citrovorum* activity, under the experimental conditions, at the same rate regardless of whether the substrate was I, II or III. The values presented were obtained using synthetic 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid (leucovorin) as a standard. Ascorbic acid was added to the flasks (10 mg./flask) to augment the synthesis of *L. citrovorum* activity.<sup>2</sup>

TABLE I

*L. Citrovorum* ACTIVITY SYNTHESIZED BY PTEROYLGLUTAMIC ACID DEFICIENT CHICK LIVER TISSUE<sup>a</sup>

None	Substrate		
	I 10 $\gamma$ /flask	II $\approx$ 10 $\gamma$ I/flask	III $\approx$ 10 $\gamma$ I/flask
0.12 $\pm$ 0.06	3.78 $\pm$ 0.51	3.50 $\pm$ 0.25	3.60 $\pm$ 0.34

<sup>a</sup> Values are the average of six experiments and are expressed as  $\gamma$  of leucovorin per g. liver slices, fresh wt., present after incubation. This amount of I is far in excess of that needed to saturate the system in respect to I. The standard error is included.

Bioautographic analyses of the total *L. citrovorum* activity synthesized under these conditions were carried out. The modified procedure of Winsten and Eigen<sup>4</sup> reported previously<sup>5</sup> was employed. Under the experimental conditions two zones of growth were consistently observed. The faster moving spot had the same  $R_f$  value and could not be separated chromatographically from leucovorin. Both zones of growth were obtained regardless of whether the substrate was I, II or III.

These observations indicate that I, II and III apparently have a common metabolic pathway in the synthesis of *L. citrovorum* activity. It is furthermore indicated that more than one substrate possessing activity for *L. citrovorum* is synthesized by chick liver tissue.

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(2) C. A. Nichol and A. D. Welch, *Proc. Soc. Expt. Biol. Med.*, **74**, 52 (1950).

(3) L. S. Dietrich, W. J. Monson and C. A. Elvehjem, *ibid.*, **77**, 93 (1951).

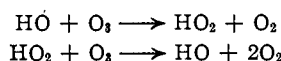
(4) W. A. Winsten and E. Eigen, *J. Biol. Chem.*, **184**, 155 (1950).

(5) L. S. Dietrich, W. J. Monson, H. Gwoh and C. A. Elvehjem, *ibid.*, **184**, 549 (1952).

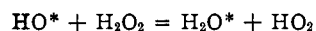
EVIDENCE FOR THE EXCHANGE OF HYDROXYL  
RADICAL WITH WATER<sup>1</sup>

Sir:

Other work has shown that the reaction of H<sub>2</sub>O<sub>2</sub> and O<sub>3</sub> produces a powerful 1e<sup>-</sup>-oxidizing agent which reacts with O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> as well as with less reactive substances such as Cl<sup>-</sup>, Br<sup>-</sup>, HOAc, etc.<sup>2</sup> It has also been shown that the same species is generated by the reaction of HCOOH with O<sub>3</sub>.<sup>3</sup> This species has been described as the hydroxyl radical, and the decomposition of O<sub>3</sub> which it catalyzes has been formulated<sup>2,4</sup> as taking place by the steps



This reaction scheme suggests a simple tracer experiment for testing exchange of HO and H<sub>2</sub>O, since the hydroxyl oxygen is converted to O<sub>2</sub>, which is known not to exchange readily with H<sub>2</sub>O. Hydrogen peroxide chemistry offers no similar convenient means of studying the exchange, since the hydroxyl oxygen in reaction with H<sub>2</sub>O<sub>2</sub> is presumably converted to water.



This communication presents some results on the exchange of O<sub>3</sub> with H<sub>2</sub>O induced by the reaction with H<sub>2</sub>O<sub>2</sub>. In all experiments ozonized oxygen of normal isotopic composition ( $N$  = mole fraction of O<sup>18</sup> = 2.000  $\times$  10<sup>-3</sup>; all isotopic compositions quoted have been normalized to this value for  $N^0$ ) was left in contact with a liquid phase containing water enriched in O<sup>18</sup> ( $N$  = 14.6  $\times$  10<sup>-3</sup>). The ratio of gas volume to liquid was approximately 5. After a time, the gas was removed, dried, any residual O<sub>3</sub> was decomposed and the isotopic composition determined. In an experiment on the direct exchange of O<sub>3</sub> with water, ozonized oxygen at atmospheric pressure, 7% O<sub>3</sub>, was left in contact with enriched water, 0.04  $M$  in HClO<sub>4</sub> for 5 days. The isotopic composition of the gas remained unchanged<sup>5</sup> at 2.000  $\times$  10<sup>-3</sup>. In a typical experiment with H<sub>2</sub>O<sub>2</sub> present, all conditions were the same except the liquid contained 9  $\times$  10<sup>-4</sup>  $M$  H<sub>2</sub>O<sub>2</sub> (normal isotopic composition,  $N$  = 1.997  $\times$  10<sup>-3</sup>). After 3 days, during which time 40% of the O<sub>3</sub> disappeared and 30% of the H<sub>2</sub>O<sub>2</sub>, the isotopic composition of the gas was found to be 2.050  $\times$  10<sup>-3</sup>.

The results quoted correspond to the exchange of about one-tenth of the oxygen contained in the O<sub>3</sub> which has decomposed. By the mechanism for decomposition which has been suggested, a maximum exchange of one-sixth of the O<sub>3</sub> oxygen can be expected.

It should be stressed that neither the earlier data nor the present data prove that the intermediate in question is HO. The present exchange data provide additional strong evidence however. H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub> and O<sub>3</sub> do not exchange at all rapidly with water.

(1) This research is supported by Office of Naval Research under contract N6-ori-02026. The funds for the purchase of the mass spectrometer were supplied by the Atomic Energy Commission under contract At(11-1)-92.

(2) H. Taube and W. C. Bray, *THIS JOURNAL*, **62**, 3357 (1940).

(3) H. Taube, *ibid.*, **63**, 2453 (1941).

(4) J. Weiss, *Trans. Faraday Soc.*, **31**, 1547 (1934).

(5) In alkaline solution extensive exchange of ozone and water does take place.

Of the radicals HO, HO<sub>2</sub> and HO<sub>3</sub>, rapid exchange seems possible only for HO.

More complete experimental results will be presented in a later report, containing also data on related systems.

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### TRACER STUDIES ON SOME REACTIONS OF THIOSULFATE AND TETRATHIONATE

Sir:

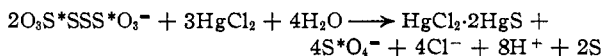
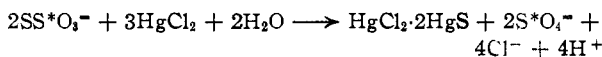
The oxidation of thiosulfate to tetrathionate with iodine, the reduction of tetrathionate to thiosulfate with sulfide, and the decomposition of both thiosulfate and tetrathionate with mercuric chloride in presence of bisulfite and excess formaldehyde have been studied with the aid of S<sup>35</sup>.

In order to separate the products of the reactions with mercuric chloride, three successive precipitations were made: HgCl<sub>2</sub>·2HgS (from thiosulfate) and HgCl<sub>2</sub>·2HgS + S (from tetrathionate) were precipitated in the cold by means of a large excess of concentrated buffered mercuric chloride and addition of some ammonia after one hour (I), sulfate was precipitated, also in the cold, with acetic acid and barium chloride (II), finally the protected bisulfite was oxidized with potassium hypobromite and precipitated as barium sulfate (III). Radioactive contamination of III was excluded by an intermediate scavenging operation which consisted of the addition of inactive thiosulfate or tetrathionate, mercuric chloride and barium chloride. These intermediate precipitates were checked to be practically inactive.

If thiosulfate labeled at the central S-atom was treated in this way the activity distribution was: 1% in I, 95% in II, none in III.

If the same thiosulfate was titrated to tetrathionate with iodine, and analyzed in the same manner, again 1% was found in I, and 95% in II, but this time 2-3% entered into III.

Saved for the infrequent side-reaction which caused the formation of radioactive sulfite during the decomposition of tetrathionate, the reactions may be assumed to proceed according to



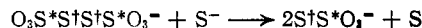
If tetrathionate, obtained by titration of the same thiosulfate with iodine, was reduced immediately with inactive sulfide (in presence of inactive bisulfite and excess formaldehyde), the sulfur formed was inactive. The filtrates from this reaction were analyzed with mercuric chloride both directly, and after they had been titrated back to tetrathionate. In the first case the activity distribution was found to be: 1% in I, 96% in II, 1-2% in III; in the second case: 1% in I, 95% in II and 3% in III.

The activities found in all fractions I may well

be introduced into the thio-S of the thiosulfate by side-reactions during its formation.

If tetrathionate was prepared from thiosulfate labeled at the thio-S, 2-3% of the total activity was always found in the solid sulfur; if inactive tetrathionate was reduced with active sulfide, 97% was found in the sulfur.

The results indicate that, save for a minor side-reaction, the reduction of tetrathionate with sulfide proceeds according to



This investigation represents part of the research program of the Foundation for Fundamental Research of Matter (F. O. M.). It was performed with the financial aid of the Netherlands Organization for pure Research (Z. W. O.).

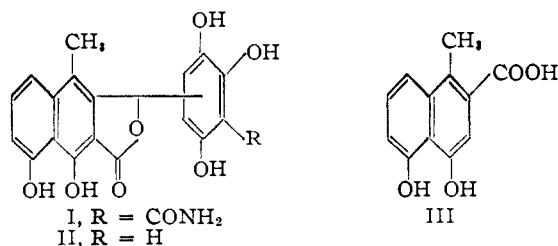
INSTITUUT VOOR KERNFYSISCH ONDERZOEK  
18 OOSTERRINGDIJK, AMSTERDAM HERMAN B. V. D. HEIJDE  
THE NETHERLANDS A. H. W. ATEN, JR.

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### TERRAMYCIN. V. STRUCTURE OF TERRINOLIDE. AN ACID DEGRADATION PRODUCT OF TERRAMYCIN

Sir:

Among the products formed by the degradation of terramycin<sup>1</sup> in dilute hydrochloric acid at elevated temperatures is terrinolide (I),  $pK_{a1} = 4.6$ ,  $pK_{a2} = 7.5$  (dimethylformamide-water);  $[\alpha]_D -16.0^\circ$  (*c* 1% in 1:1 methanol-0.1 *N* hydrochloric acid). *Anal.* Calcd. for C<sub>20</sub>H<sub>15</sub>NO<sub>8</sub>: C, 60.45; H, 3.81; N, 3.53. Found: C, 60.46; H, 4.10; N, 3.52. On hydrolysis in hot 12 *N* sulfuric acid, terrinolide loses ammonia and carbon dioxide to yield a nitrogen-free, optically-inactive compound, decarboxamidoterrinolide (II),<sup>2</sup>  $pK_{a1} = 4.7$ ,  $pK_{a2} = 10.2$  (dimethylformamide-water). *Anal.* Calcd. for C<sub>19</sub>H<sub>14</sub>O<sub>7</sub>: C, 64.41; H, 3.98; C-methyl, 4.25. Found: C, 64.10; H, 4.41; C-methyl, 3.82. Pentamethyldecarboxamidoterrinolide: m.p. 152-153°, *Anal.* Calcd. for C<sub>24</sub>H<sub>24</sub>O<sub>7</sub>: C, 67.91; H, 5.70; CH<sub>3</sub>O, 36.55. Found: C, 67.85; H, 5.74; CH<sub>3</sub>O, 35.95. Terrinolide and decarboxamidoterrinolide have been assigned structures I and II, respectively.



Alkali fusion of II yields 1,8-dihydroxy-4-methyl-3-naphthoic acid (III).<sup>3</sup> I, II and III enhance the

(1) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, *THIS JOURNAL*, **73**, 4211 (1951).

(2) Terrinolide and decarboxamidoterrinolide were originally assigned the formulas C<sub>19</sub>H<sub>17</sub>NO<sub>8</sub> and C<sub>18</sub>H<sub>15</sub>O<sub>8</sub>, respectively, in our first communication (R. Pasternack, P. Regna, R. Wagner, A. Bavyly, F. Hochstein, P. Gordon and K. Brunings, *THIS JOURNAL*, **73**, 2400 (1951)). The formation of stable solvates and a tendency of these compounds to decompose under conditions of molecular weight determination complicated the assignment of the molecular formulas.

(3) F. A. Hochstein, *et al.*, to be published.