

UTILIZATION OF PTEROYLGLUTAMIC ACID
CONJUGATES IN THE *IN VITRO* SYNTHESIS OF
L. CITROVORUM ACTIVITY¹

Sir:

The demonstration that liver slices convert synthetic pteroylglutamic acid (I) to a substance(s) possessing microbiological activity for *L. citrovorum*² 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 Welch² and employing the modifications reported previously³ 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.²

TABLE I

L. Citrovorum ACTIVITY SYNTHESIZED BY PTEROYLGLUTAMIC ACID DEFICIENT CHICK LIVER TISSUE^a

| None | Substrate | | |
|-----------------|-------------------------|-------------------------------------|--------------------------------------|
| | I 10 γ /flask | II \approx 10 γ I/flask | III \approx 10 γ I/flask |
| 0.12 \pm 0.06 | 3.78 \pm 0.51 | 3.50 \pm 0.25 | 3.60 \pm 0.34 |

^a Values are the average of six experiments and are expressed as γ 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 Eigen⁴ reported previously⁵ 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.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

L. S. DIETRICH
W. J. MONSON
C. A. ELVEHJEM

RECEIVED MAY 16, 1952

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by funds supplied by Swift and Co., Chicago, Ill., and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. We are indebted to Merck and Co., Rahway, N. J., for some of the crystalline vitamins, to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for synthetic pteroylglutamic acid, synthetic pteroyldiglutamic acid and synthetic pteroyltriglutamic acid.

(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).

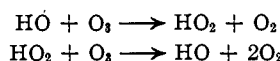
(4) W. A. Winsten and E. Eigen, *J. Biol. Chem.*, **184**, 155 (1950).

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

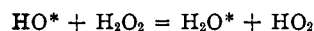
EVIDENCE FOR THE EXCHANGE OF HYDROXYL
RADICAL WITH WATER¹

Sir:

Other work has shown that the reaction of H₂O₂ and O₃ produces a powerful 1e⁻-oxidizing agent which reacts with O₃, H₂O₂ as well as with less reactive substances such as Cl⁻, Br⁻, HOAc, etc.² It has also been shown that the same species is generated by the reaction of HCOOH with O₃.³ This species has been described as the hydroxyl radical, and the decomposition of O₃ which it catalyzes has been formulated^{2,4} as taking place by the steps



This reaction scheme suggests a simple tracer experiment for testing exchange of HO and H₂O, since the hydroxyl oxygen is converted to O₂, which is known not to exchange readily with H₂O. Hydrogen peroxide chemistry offers no similar convenient means of studying the exchange, since the hydroxyl oxygen in reaction with H₂O₂ is presumably converted to water.



This communication presents some results on the exchange of O₃ with H₂O induced by the reaction with H₂O₂. In all experiments ozonized oxygen of normal isotopic composition (N = mole fraction of O¹⁸ = 2.000×10^{-3} ; 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 O¹⁸ ($N = 14.6 \times 10^{-3}$). The ratio of gas volume to liquid was approximately 5. After a time, the gas was removed, dried, any residual O₃ was decomposed and the isotopic composition determined. In an experiment on the direct exchange of O₃ with water, ozonized oxygen at atmospheric pressure, 7% O₃, was left in contact with enriched water, 0.04 M in HClO₄ for 5 days. The isotopic composition of the gas remained unchanged⁵ at 2.000×10^{-3} . In a typical experiment with H₂O₂ present, all conditions were the same except the liquid contained $9 \times 10^{-4} M$ H₂O₂ (normal isotopic composition, $N = 1.997 \times 10^{-3}$). After 3 days, during which time 40% of the O₃ disappeared and 30% of the H₂O₂, the isotopic composition of the gas was found to be 2.050×10^{-3} .

The results quoted correspond to the exchange of about one-tenth of the oxygen contained in the O₃ which has decomposed. By the mechanism for decomposition which has been suggested, a maximum exchange of one-sixth of the O₃ 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. H₂O₂, O₂ and O₃ 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.