thea A. Ageledis of the Hoffman–LaRoche Co., Inc., for carrying out the Van Slyke determinations and to Mr. William Saschek for the microanalyses. Thanks are also due to Dr. Richard M. Adams for his help in carrying out some of the experiments.
CHICAGO, ILLINOIS

COMMUNICATIONS TO THE EDITOR

EVIDENCE FOR THE EXCRETION OF FORMIMINOGLUTAMIC ACID FOLLOWING FOLIC ACID ANTAGONIST THERAPY IN ACUTE LEUKEMIA

Sir:

Rats on a diet deficient in folic acid (FA) excrete a heat- and alkali-labile derivative of glutamic acid[1] presumably identical with N-formiminoglutamic acid (I) (α-formamidinoglutaric acid).[2] It was suggested recently that with a suitable extract of mammalian liver the formimino group of (I) may be transferred to tetrahydro-FA.[3] Recent publications[4,5] give evidence that the degradation, of formiminoglycine is also FA dependent. Preliminary microbiological and chromatographic evidence shows that a compound so far indistinguishable from synthetic (I) is excreted in the urine of children with acute leukemia during FA antagonist therapy.

The glutamic acid excretion patterns of various individuals are in Table I. The microbiological response to the unheated urine samples should be a measure of the glutamine and free glutamic acid present in the urine, but not of (I) which is microbiologically inactive. In contrast, the microbiological response to heated urine samples should be a measure of any glutamic acid originally present plus glutamic acid arising from (I) during heating. The glutamine present in unheated urine was destroyed by the heating process and did not contribute to the microbiological activity of the heated samples. Glutamic acid activity in urine from normal individuals could be detected only when the samples were unheated and paper chromatography indicated that the activity was due to glutamine. In contrast, urine specimens from two children with acute leukemia being treated with N - [4 - {N - (2,4 - diamino - 6 - pteridyl) - methyl]-N-methylamino}-benzoyl]-glutamic acid (Methotrexate) contained glutamic acid activity when the samples were either heated or unheated. Moreover, when the antagonist was discontinued, a marked drop in the glutamic acid content of the heated urine samples was observed. These observations could be explained by assuming that (I) was being excreted and that its presence may be associated with the degree of FA deficiency induced by the antagonist. An alternate explanation would be that these patients excreted glutamic acid rather than glutamine. However, evidence was found favoring the first of these two explanations as follows.

Synthetic (I) was separated by paper chromatography from glutamic acid (Strip A and B, Fig. 1). One hundred ml. of urine (representing 16% of the 24-hour total from Case 1, during Methotrexate therapy) was treated with charcoal (see footnote, Table I), filtered, and a mercury precipitation performed as described elsewhere[6] for the isolation of (I). The precipitate was decomposed with hydrogen sulfide, filtered and the filtrate was lyophilized and reconstituted with 10 ml. water; 80% of the original glutamic acid activity in the heated microbiological assay was recovered. The

TABLE I

EXCRETION OF GLUTAMIC ACID ACTIVITY IN NORMAL INDIVIDUALS AND CHILDREN WITH ACUTE LEUKEMIA TREATED WITH 4-AMINO-10-METHYLPTEROYLGLUTAMIC ACID (METHOTREXATE)

Description of individual studied	Glutamic acid excreted,[a] mg./day: Urine unheated[b]	Urine heated[b]
Normal individuals		
12 yr. old boy	59	None detected
6 yr. old boy	60	None detected
Adult female	91	None detected
Acute Leukemia[c]		
Case 1, 12 yr. old boy, on Methotrexate 2.5 mg. per day, 30 days	38	45
Case 1, off Methotrexate 10 days	55	20
Case 2, 14 yr. old girl, on Methotrexate 5 mg. per day, 14 days	35	39
Case 2, off Methotrexate 3 days	12.5	3.5

[a] Determined with *Lactobacillus arabinosus* 17-5 by the method of Henderson and Snell[7]; sensitivity about 5γ glutamic acid per ml. [b] Unheated test samples were sterilized by Seitz filtration and added aseptically to sterile cooled media. Heated samples were autoclaved for 15 minutes at 15 lb. pressure at *p*H 7, then added aseptically to sterile media. [c] Urine from these cases was adjusted to *p*H 2–3 and stirred with 2% charcoal (Darco G-60) for 30 minutes, filtered, and the filtrate brought to *p*H 7 for the microbiological assay. Such a procedure effectively removed Methotrexate present in urine; control experiments showed such urine contained no growth inhibiting substances.

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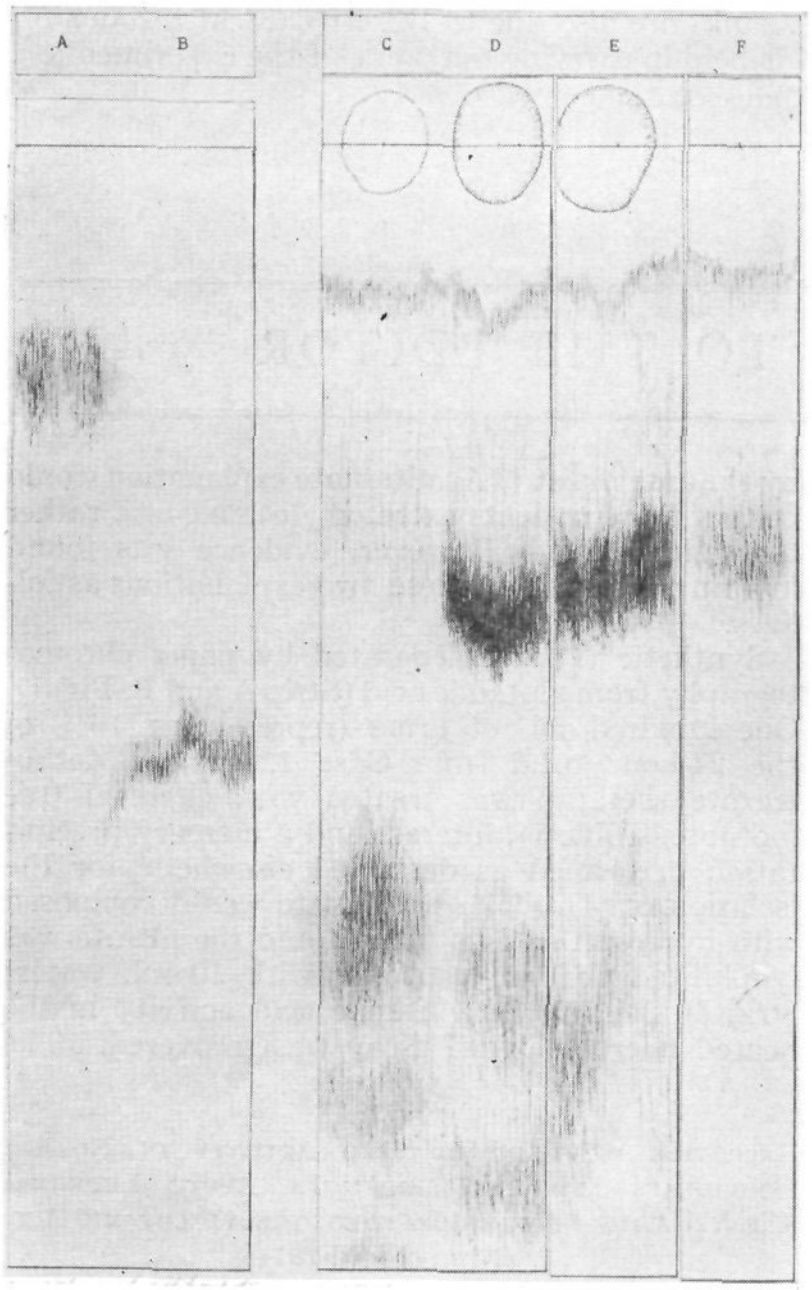

Fig. 1.—Chromatographic evidence for the presence of formiminoglutamic acid in urine following folic acid antagonist therapy in acute leukemia: solvent system: 100 g. phenol + 20 ml. water containing 6.3% sodium citrate, 3.7% monobasic potassium phosphate, and 0.5% ascorbic acid. Chromatographic conditions: descending technique, 36 hr., 25–30°; development with ninhydrin: Strip A, 20 γ glutamic acid; Strip B, 40 γ formiminoglutamic acid (strip first exposed to ammonia vapor, 1 hr., then aerated, then sprayed with ninhydrin); Strip C, 0.04 ml. of urine eluate (see text); Strip D, 0.04 ml. urine eluate autoclaved 10 min., 15 lb. pressure, *p*H 7.5, then chromatographed; Strip E, urine eluate adjusted to *p*H 12 and held 24 hr. at 25°, then chromatographed; Strip F, 20 γ glutamic acid, control for chromatograms, C, D, and E.

concentrate (0.7 ml.) was next applied in 0.02 ml. amounts across a sheet of Whatman #1 paper (18.5 × 11.5) and developed in the solvent system described in Fig. 1 for 36 hours. Separate chromatograms of glutamic acid and (I) served as controls which were visualized by spraying with ninhydrin, and that area of the urine chromatogram coincident with (I) was cut out and eluted *with water (final volume, 0.5 ml.). The eluate* was devoid of microbiological activity for glutamic acid unless autoclaved and contained glutamic acid activity equivalent to 200 γ/ml. (see Table I). When the eluate was either autoclaved or let stand for 24 hours at 25° at *p*H 12, the concomitant formation of glutamic acid could be demonstrated by paper chromatography (Fig. 1, Strips C, D, E, and F). The properties of the glutamic acid derivative present in the urine eluate were shown by (I). Comparable treatment of normal urine did not yield any material containing alkali- or heat-labile glutamic acid activity. Work on the isolation of the glutamic acid precursor from appropriate urine specimens is in progress to confirm the above findings.

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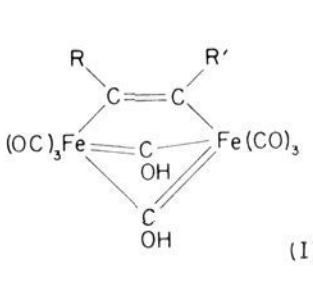
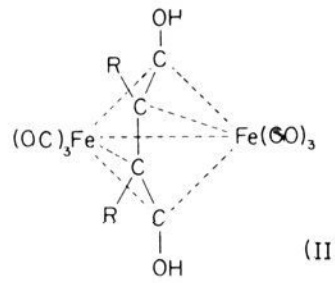
THE STRUCTURE OF THE BRIDGED IRON COMPLEXES FROM IRON HYDROCARBONYL AND ACETYLENES

Sir:

The complex $Fe_2C_{10}H_4O_8$,[1] obtained from acetylene and the monoanion of iron carbonyl hydride, has been discussed recently and tentatively depicted as (I, R = R′ = H).[2] The propyne and



(I)



(II)

hex-1-yne analogs were described in 1955,[3] but analytical difficulties prevented early publication of our results. We now present evidence for an alternative formula (II). Analogous complexes can be obtained from acetylene, propyne, and but-2-yne by essentially similar methods; they have the same composition $H_2[RC{\equiv}CRFe_2(CO)_8]$, and show similar chemical and spectroscopic properties. However, mild acetylation converts the acetylene complex into a diacetate, while the propyne complex forms a monoacetate and the but-2-yne complex is unchanged. All yield diacetates under more vigorous conditions; that from propyne is converted by hot methanol into an isomeric monoacetate. Chloroacetic anhydride converts the two monoacetates into different acetyl-chloroacetyl derivatives, m.ps. 107–108° and 89–90°, respectively. We conclude (a) that in the propyne complex the two hydroxyl groups are not placed symmetrically with respect to the propyne molecule [as they would be in (I)]; and (b) that the hydroxyl groups are sufficiently close to the acetylene residue to suffer appreciable steric hindrance from even a methyl substituent on the latter.

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