At present calcium *d*L-leucovorin (V) has not been obtained in a pure state; however, a fraction has been obtained with a high rotation, lowered microbiological activity, and polarographic activity[4] identical with I (see Table I). The purification of V is now in progress.

TABLE I

	I[a]	III	IV[e]	V
$[\alpha]_D$	+14.26	−15.1		+28.3
c, %[b]	3.42	1.82		3.53
CF assay,[c]	800	1640	1340	576
after acid	40	40	40	31
PGA assay,[d]	550	1070	890	247
after acid	1050	970	700	721

[a] As the calcium salt. [b] Concentration in water calculated for the anhydrous calcium salt. [c] In γ/mg., using anhydrous I as the standard for *Le. citrovorum* 8081. [d] In γ/mg., with II as the standard for *S. faecalis* R. [e] The solution of citrovorum factor (1 mg./ml.) was furnished by Dr. John C. Keresztesy.

CALCO CHEMICAL DIVISION
AMERICAN CYANAMID COMPANY
BOUND BROOK, N. J., AND
LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

DONNA B. COSULICH
JAMES M. SMITH, JR.
HARRY P. BROQUIST

RECEIVED JULY 7, 1952

HIGH ENERGY HELIUM-ION IRRADIATION OF FORMIC ACID IN AQUEOUS SOLUTION[1]

Sir:

Several years ago Fricke, Hart and Smith[2] measured the amounts of hydrogen and carbon dioxide formed by X-ray induced reactions in aqueous solutions of formic acid over a wide range of *p*H values and solute concentrations. To account for the observed gas yields, they suggested that under certain conditions both oxalic acid and formaldehyde may be formed in addition to hydrogen and carbon dioxide. Recently, as part of a general study[3,4] of the radiation induced synthesis of organic substances in aqueous solutions, we have found that oxalic acid, formaldehyde and at least eight other organic compounds are produced by high energy helium-ion bombardment of hydrogen-saturated aqueous formic acid solutions. The present preliminary communication describes briefly the experimental techniques and observations. A more detailed report of this work will be forthcoming.

Air-free aqueous solutions of C^{14}-labelled formic acid[5] were irradiated in all-glass target cells with the 40 Mev. helium-ion beam of the 60-inch cyclotron at the Crocker Laboratory. Hydrogen gas was bubbled through the solution during exposure and then passed through dilute sodium hydroxide solution to recover carbon dioxide which was subsequently assayed as $BaC^{14}O_3$. After irradiation the target solution was distilled to dryness *in vacuo* at room temperature. The $HC^{14}HO$ in the distillate was isolated and assayed as the methone-formaldehyde derivative.[3] A two-dimensional paper chromatogram of the non-volatile fraction was prepared[6] and radioautographed. One major spot typical of oxalic acid and eight other spots of lesser intensity could be seen in the original radioautogram. The region containing the major part of the activity was eluted with 0.1 N hydrochloric acid. The activity in an aliquot of this solution co-precipitated quantitatively on lanthanum oxalate after repeated washing and recrystallization from dilute nitric acid. To further identify this major non-volatile product as oxalic acid a second aliquot was cochromatographed with added oxalic acid on a silica column by a partition chromatography method similar to one recently reported.[7] An exact correspondence of oxalic acid titer and C^{14} activity in the eluant was observed. A 300-microcurie sample of the $HC^{14}OOH$ used in these experiments was analyzed in exactly the same way as the bombarded solutions; the control showed no activity other than $HC^{14}OOH$. Decay of the activity in the products could not be detected over a period of several months.

Each of the 10 ml. target solutions contained 300 microcuries of $HC^{14}OOH$ and were 0.009 N in total formic acid. The helium ions incident on the solution had an energy of 35 Mev. Bombardments were made at a beam current of 0.10 microampere for a period of 3.0 minutes to give a total exposure of 0.005 microampere hour. Yield data are summarized in Table I.

TABLE I

Product	G (molecules/100 e.v.) 1	2
Carbon dioxide		0.75
Oxalic acid	0.023	0.027
Formaldehyde	0.61×10^{-3}	0.56×10^{-3}

We wish to thank Mrs. Jeanne Gile-Melchert and Mrs. Harriet Powers for their assistance in the column separations, Mr. Boyd Weeks for his help in the target assembly, and the staff of the 60-inch cyclotron at the Crocker Laboratory for the bombardments.

---

(1) The work reported in this paper was performed under Contract W-7405-eng-48A with the United States Atomic Energy Commission.

(2) H. Fricke, E. J. Hart and H. P. Smith, *J. Chem. Phys.*, **6**, 229 (1938).

(3) W. M. Garrison, D. C. Morrison, J. G. Hamilton, A. A. Benson, and M. Calvin, *Science*, **114**, 416 (1951).

(4) W. M. Garrison, and G. K. Rollefson, *Discussions Faraday Soc.*, **11**, (in press) (1952).

(5) We wish to thank Dr. Bert M. Tolbert for supplying the $HC^{14}OOH$ used in these experiments.

(6) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Hass and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

(7) W. A. Bulen, J. E. Varner and R. C. Burrel, *Anal. Chem.*, **24**, 187 (1952).

CROCKER LABORATORY,
UNIVERSITY OF CALIFORNIA
RADIATION LABORATORY
DIVISIONS OF MEDICAL PHYSICS
EXPERIMENTAL MEDICINE AND RADIOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY AND SAN FRANCISCO, CALIFORNIA

WARREN M. GARRISON
DONALD C. MORRISON
HERMAN R. HAYMOND
JOSEPH G. HAMILTON

RECEIVED JULY 14, 1952

DIFFERENCES IN THE QUANTITATIVE AMINO ACID COMPOSITION OF INSULINS ISOLATED FROM BEEF, PORK AND SHEEP GLANDS

Sir:

Recently insulin preparations from different animal species have been successfully fractionated by countercurrent distribution.[1] Each preparation appeared to contain a major, or A, component

---

(1) E. J. Harfenist and L. C. Craig, THIS JOURNAL, **74**, 3083 (1952).