At present calcium *dL*-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.

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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.

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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

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and varying amounts of minor components. In the system studied the partition ratios of the major components of beef, pork and sheep insulins appeared to be identical within the experimental error, but this by no means proves that these components are identical, particularly with a solute of the size and complexity of insulin. It indicates only that their physical properties are remarkably similar. From the standpoint of a critical evaluation of the experimental methods available for separating intact proteins and in order to find out whether or not the purity of a given preparation can be established with any degree of reliability by countercurrent distribution, it is important to compare these purified A components from the different species in the most searching manner. We have therefore compared their quantitative amino acid compositions by ion exchange chromatography.[2] Striking differences have been found.

Analyses of products from hydrolyses carried out for 24 hours have shown that there are six amino acids which are present in different amounts in insulins prepared from the three species mentioned. These are given in Table I. Otherwise the amino acid compositions of the three preparations were identical. Minimum molecular weights of 5734, 5778 and 5704 were calculated for beef, pork and sheep insulins, respectively, from the amino acid compositions using the closest integral values for the number of residues of each amino acid. Since molecular weight determination by the method of partial substitution[3] on beef insulin has indicated a value in the range of 6,000, these minimum values were used as a basis for the numbers of residues of the six amino acids in Table I. All of the other amino acids are also present in approximately molar ratios when these molecular weights are used. Recent studies[4] by physical methods, however, have indicated a value for the molecular weight in the range of 12,000.

TABLE I

Amino acid	Beef: Amino acid residue per 100 g. protein, g.	Beef: Residues	Pork: Amino acid residue per 100 g. protein, g.	Pork: Residues	Sheep: Amino acid residue per 100 g. protein, g.	Sheep: Residues
Serine[a]	4.38	2.89	4.21	2.79	3.16	2.07
Threonine[a]	1.71	0.97	3.10	1.77	1.71	0.96
Glycine	3.92	3.94	3.89	3.94	4.70	4.70
Alanine	3.60	2.91	2.67	2.17	3.72	2.99
Valine[b]	8.10	4.68	6.31	3.68	8.34	4.80
Isoleucine[b]	1.30	0.66	3.02	1.54	1.36	0.69

[a] Corrected for decomposition during hydrolysis. [b] In the case of beef insulin the amounts of valine and isoleucine increased to molar proportions when the hydrolysis periods were increased to 48 hours and 96 hours, respectively.

Sanger[5] has obtained evidence by paper chromatography that four of the amino acids in Table I may be present in different amounts in beef, pork and sheep insulins. Lens and Evertzen[6] supported his conclusions. Other than these results, all studies based on physical methods, physiological activity and immunological specificity[7] have not shown species differences in purified insulins. Therefore the quantitative differences reported here must result in very slight physical and physiological differences. Species differences are well known to occur in the larger proteins. The results reported here suggest that similar species differences will be found in the smaller protein or peptide hormones.

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RECEIVED JULY 7, 1952

THE ACTION OF CARBOXYPEPTIDASE ON OVALBUMIN

Sir:

It has been shown by Desnuelle and Casal[1] and also by Porter[2] that the ovalbumin molecule contains no N-terminal amino acid residues according to the Sanger dinitrofluorobenzene (DNFB) method.[3] This finding might be accounted for in several ways: (I) end-to-end cyclization of the ovalbumin molecule; (II) internal cyclization of the N-terminal portion of the molecule; (III) masking of the α-amino groups due to combination with the non-protein moiety of the molecule (carbohydrate[4] or phosphate[5]); (IV) steric hindrance limiting the reactivity of free α-amino groups. If (I) were correct it should not be possible to demonstrate the presence of any free C-terminal residues. However, the present experiments in which the substrate specificity of carboxypeptidase[6-10] has been utilized indicate the presence of alanine as a C-terminal residue in ovalbumin.

A 3% solution of 4 times recrystallized ovalbumin was incubated at *p*H 7.4 with a suspension of commercial crystalline carboxypeptidase (Worthington) at a concentration of 0.08–0.15 mg. per ml. In order to rule out contaminating proteinase activities the experiments were repeated after three additional crystallizations of the enzyme.[11,12] No difference in results was noted. Aliquots of the reaction mixture were removed at intervals and adjusted to the isoelectric point of ovalbumin (*p*H 4.7) by the addition of 0.01 *N* HCl. Four volumes of absolute ethanol were then added and the alcoholic suspension was boiled for 5–10 min. The coagulated protein was removed by centrif-

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