with silk sutures, but the level of recoverable thrombin was not elevated and no clot was found post mortem. In rabbits Nos. 6 and 7 the veins were merely exposed and no clot was found on autopsy. One of these animals (No. 6) showed an elevated level of recoverable thrombin on the third postoperative day, which may reflect the occurrence of minute thrombi associated with the surgical procedure.

Unlike recoverable thrombin, prothrombin levels remained normal throughout the postoperative period in each animal.

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Reduction of Carbon Dioxide in Aqueous Solutions by Ionizing Radiation[1]

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The question of the conditions under which living matter originated on the surface of the earth is still a subject limited largely to speculation. The speculation has a greater chance of approaching the truth when it includes and is based upon the ever wider variety of established scientific fact. One of the purposes of the observation reported herein is to add another fact that might have some bearing upon this interesting question.

One of the most popular current conceptions is that life originated in an organic milieu (*1–5*). The problem to which we are addressed is the origin of that organic milieu in the absence of any life. It appeared to us that one source—if not the only one—of reduced carbon compounds in complex arrangements might be the interaction of various high-energy radiations with aqueous solutions of inorganic materials, particularly carbon dioxide, and nitrogenous compounds such as ammonia and nitrogen, since it appears that these compounds were the commoner forms in which the essential elements were to be found on the primordial earth (*6, 7*).

Although it has long been known that high-energy radiations can cause organic decomposition and oxidation, it seemed useful to us to demonstrate that conditions could be found in which high-energy radiations could induce the reduction with water of carbon dioxide and the ultimate creation of polyatomic molecules (other than simple polymerization of monomers) of carbon, oxygen, hydrogen, and nitrogen.

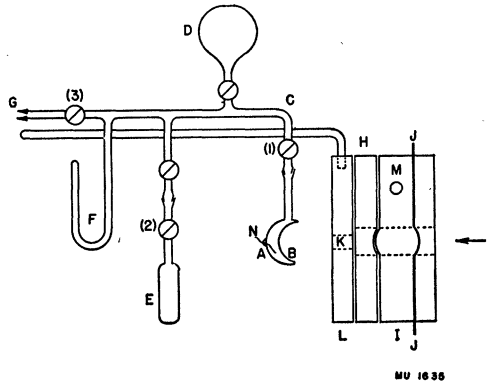

FIG. 1.

The general technique employed was to bombard air-free aqueous solutions of C^{14}-labeled CO_2 in a closed system with and without the addition of ferrous sulfate. The bombardments were made using the 40-mev helium ion beam of the 60-inch cyclotron at Crocker Laboratory. To detect the amount and nature of the reduction products, chemical separations were made on the bombarded solution after the addition of carrier amounts of formic acid, formaldehyde, and methyl alcohol. These were separated as solid derivatives and assayed for C^{14} activity. In most of the bombardments 1 mc of 5–9% C^{14}-labeled CO_2 was used. This made it possible to detect the reduction of approximately one part in 10^6.

A diagram of the target assembly is shown in Fig. 1. The aqueous solutions were bombarded in an all-glass target cell (*A*) which consisted essentially of a 50-ml Pyrex flask, one side of which was drawn in to give a window (*B*) having an average thickness of approximately 5 mil over the bombarded area. The cell had a volume of 12 ml. It was connected to a glass manifold (*C*), which, in turn, was connected through stopcocks to a 100-ml product gas storage bulb (*D*) to a 25-ml CO_2 reservoir (*E*) to a mercury manometer (*F*), and to an outlet (*G*) through which the entire system could be evacuated. The assembly was supported on a bracket (*H*), which was fastened to the bell-jar–type target (*I*). The helium ion beam was brought out of the cyclotron vacuum through a 1.5-mil aluminum foil (*J*) and was delimited in cross section by the aperture (*K*) in plate (*L*). The target window was cooled by means of an air stream, which entered at (*M*) and emerged through the aperture (*K*). The beam current was monitored through the electrode (*N*). With the all-glass target cell it was necessary, because of the nonuniform thickness of the window, to calculate the number of ion-pairs produced from the amount of Fe^{+2} oxidation, assuming the same ion-pair yield for this reaction in the glass cell as was obtained in the cell having the platinum window. With the latter tar-

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TABLE 1

Bombardment	(1)	(2)	(3)	(4)	(5)
Cell window	Glass	Glass	Glass	Platinum	Glass
Solute	$C^{14}O_2 + FeSO_4$	$C^{14}O_2 + FeSO_4$	$C^{14}O_2$	$CO_2 + FeSO_4$	$CO_2 + FeSO_4$
Vol of solution (ml)	12.0	12.0	12.0	12.0	12.0
Gas vol, manifold + product gas bulb (ml)	145.	145.	145.	145.	145.
Initial conc $C^{14}O_2$ in solution (M)	6.8×10^{-5}	8.2×10^{-5}	1.0×10^{-5}	8.2×10^{-5}	$\backsim 8 \times 10^{-5}$
No. CO_2 molecules dissolved in H_2O phase	5.5×10^{17}	6.7×10^{17}	8.3×10^{17}	6.2×10^{17}	$\backsim 7 \times 10^{17}$
Partial pressure of $C^{14}O_2$ in gas space (mm Hg)	2.4	2.9	3.6	2.7	
C^{14} activity (mc)	1	1	1	None	—
C^{14} in CO_2 (%)	9.0	6.0	4.8	—	—
Energy of emerging helium ions (mev)	40.	40.	40.	40.	40.
Bombardment current (μa)	0.5	0.5	0.5	0.5	0.5
Total bombardment (μa-hr)	0.75	0.042	0.042	0.13	0.13
No. ion-pairs produced in solution (assuming 32.5 eu/ip)	7.8×10^{21}	4.4×10^{20}	4.4×10^{20}	3.1×10^{21}	1.3×10^{21}
Hydrogen pressure after bombardment (mm Hg)	208.	8.	*	*	*
No. hydrogen molecules produced	1.1×10^{21}	$.43 \times 10^{20}$	—	—	—
Initial conc Fe^{+2} (M)	0.80	*	None	0.77	—
Conc Fe^{+3} after bombardment	0.23	—	—	0.10	—
No. of Fe^{+3} atoms formed	1.5×10^{21}	—	—	6.2×10^{20}	—
Ion-pair yield for Fe^{+3} formation	0.20	—	—	0.20	—
Total C^{14} activity in the HCOOH fraction (μc)	0.21	1.32	8.7×10^{-3}	Inactive	Inactive
No. CO_2 molecules reduced to HCOOH	2.2×10^{16}	1.5×10^{17}	1.2×10^{15}	—	—
Fraction of dissolved CO_2 reduced to HCOOH	0.04	0.22	1.4×10^{-3}	—	—
Ion-pair yield for HCOOH formation	2.9×10^{-6}	3.4×10^{-4}	2.8×10^{-6}	—	—
Total C^{14} activity in the HCHO fraction (μc)	5.7×10^{-3}	8×10^{-3}	Inactive	Inactive	Inactive
No. CO_2 molecules reduced to HCHO	6.4×10^{14}	9×10^{14}	—	—	—
Ion-pair yield for HCHO formation	0.82×10^{-7}	2.1×10^{-6}	—	—	—

* Not determined.

get cell, it was possible to estimate within a few per cent the energy loss of the helium ions in penetrating the 1.5-mm aluminum foil, 10 cm of air path, and the 1-mil platinum window.

The target cell was first flushed with nitrogen, then filled with triple-distilled deaerated water, or deaerated 1 M ferrous sulfate solution at a pH of approximately 3.5. The water was deaerated by boiling and then allowed to cool in a glass-stoppered vessel filled with nitrogen gas. The 1 M ferrous sulfate solutions were prepared by adding a known weight of ferrous sulfate. After the target cell was filled, it was immediately connected to the manifold, which was then evacuated until roughly 5% of the target solution had been evaporated. Stopcock (1) was then closed, and the manifold was evacuated, including the product gas storage bulb and that portion of the manifold stopcock (2) which was connected to the CO_2 reservoir containing approximately 1 mc of 5–9% C^{14}-labeled CO_2. After the evacuation was complete, the manifold was isolated by closing stopcock (3). Stopcocks (1) and (2) were then opened, and the CO_2 was allowed to equilibrate with the target solution. The target cell was then bombarded with a 0.5 μa beam of 40-mev helium ions. Bombardment data for each of the experiments are summarized in Table 1.

After bombardment, the target cell was allowed to stand for 1–2 hr to permit the induced radioactivity to decay out. Stopcocks (1) and (2) were closed, and the target cell was removed from the manifold. The solution was then treated with sulfuric acid to dissolve the ferric hydroxide, and adjusted to pH 1. The unreacted $C^{14}O_2$ was stripped with nitrogen and recovered in sodium hydroxide solution. After most of the high specific activity $C^{14}O_2$ had been removed, the solution was flushed with tank CO_2, which was discarded. A sample of the solution was withdrawn at this point for ferric ion analysis.

To the remainder of the solution formic acid, formaldehyde, and methyl alcohol carriers were added in amounts to give 100 mg of the isolated product—i.e., barium formate, methone derivative of formaldehyde, and barium carbonate prepared from the CO_2 formed on oxidation of the methyl alcohol fraction.

The pH of the solution was then adjusted to 7, and the formaldehyde and methyl alcohol were distilled *in vacuo*. The distillate was treated with methone solution in 50% excess and acidified. This precipitated the methone-formaldehyde derivative, and the methyl alcohol was separated from this mixture by a second vacuum distillation. The methyl alcohol distillate was wet-oxidized with a chromium trioxide-sulfuric acid mixture containing potassium iodate, and the evolved CO_2 was recovered as barium carbonate. In none of the bombardments was this barium carbonate fraction active.

The methone-formaldehyde precipitate was filtered off, washed, and dissolved in sodium hydroxide. The solution was acidified and the precipitate centrifuged, washed, redissolved in sodium hydroxide, and reprecipitated. This procedure was repeated, and then the methone-formaldehyde reaction product was recrystallized twice from acetone-water. A sample of the purified methone-formaldehyde product was counted.

The residue from the first distillation containing the formic acid was acidified to pH 1 and distilled *in vacuo*. The distillate was titrated to phenolphthalein end point with a saturated barium hydroxide solution after flushing with CO_2 followed by nitrogen. The precipitate of barium carbonate which formed was centrifuged off. The supernatant containing the barium formate was evaporated to approximately 0.5 ml and while warm was treated with absolute ethyl alcohol, which precipitated crystalline barium formate. This was redissolved in water and recrystallized in this manner four times.

A fraction of original solution, which had been removed for iron determination, was acidified with 6 *N* sulfuric acid and titrated with standard solution of potassium permanganate. A second fraction of this solution was reduced with sulfur dioxide and titrated with potassium permangante after the excess sulfur dioxide was removed by boiling. The Fe^{+3} concentration in the target solution was calculated from the difference in titer. In Table 1, Bombardments 1, 2, and 3 were made using the all-glass cell. Bombardment 4 was made using the cell having a 1-mil platinum window. With this cell, the helium ion beam incident on the solution had an energy of 35.8 mev. The number of ion-pairs produced in Bombardments 1, 2, and 3 were calculated, assuming that the ion-pair yield for ferric ion oxidation obtained in Bombardment 4 was also obtained using the all-glass target cells. This assumption is considered reasonable since the energy losses in the glass and platinum windows were of the same order of magnitude.

To insure that $HC^{14}OOH$ and $HC^{14}HO$ were actually produced by helium ion bombardment, the following additional control experiments were performed: (1) A sample of the original unbombarded target solution containing $C^{14}O_2$ and $FeSO_4$ was retained at approximately 30° C for 1 week and then processed in a manner identical with that used in separating the HCOOH, HCHO and CH_3OH fractions in the bombarded samples. No C^{14} activity could be detected in these fractions from the unbombarded solution, indicating that reduced C^{14} compounds were not present in the original solution or formed by a metabolic process involving mold or other organisms. (2) A blank bombardment (#5) was made without added $C^{14}O_2$; the isolated HCOOH and HCHO carriers were inactive. (3) Mass absorption curves run on active barium formate produced in the radiation reduction of $C^{14}O_2$ were identical with those obtained using known samples of active barium formate prepared chemically and having the same specific activity and counting geometry. (4) No decay could be detected in the activity of the radiation produced $HC^{14}OOH$ and $HC^{14}HO$.

An examination of Table 1 demonstrates unequivocally that it is quite possible to reduce appreciable quantities of carbon dioxide to formic acid by means of water through the agency of radiation. In fact, it appears that approximately one fourth of the dissolved carbon dioxide was reduced in Expt 2. Whether the formic acid is further reduced to formaldehyde or whether the formaldehyde has its origin in a direct reduction of carbon dioxide still remains to be demonstrated, but formaldehyde can also be produced from carbon dioxide and water under the influence of radiation.

The actual ion-pair yield is certainly not optimal even in Expt 2 in view of the large excess of the number of ion-pairs produced over the number of molecules of carbon dioxide in the solution. Presumably this reduction is achieved by means of the secondary hydrogen atoms resulting from the ionization. The actual amount of reduction observed is clearly still only the resultant of the reduction and oxidation reactions. The oxidation reaction is presumably minimized by the destruction of the hydroxyl radicals by their reaction with ferrous ion (*8–10*).

Whether carbon—carbon bonds and carbon—nitrogen bonds can be formed and more highly organized structures created under the influence of high-energy radiations is at present under investigation.

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Fungus Fruiting in Submerged Culture

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Although several species of ascomycetes have been grown in submerged liquid culture, the writer has been unable to discover any reports of perithecium formation under these conditions. Burkholder and Sinnott (*1*) studied the morphogenesis of about 150 species of fungi, including a number of ascomycetes, in shake cultures in three different nutrient solutions. In no case were perithecia reported, although this may have been due to the use of too short a culture period or to the possibility that none of these media was conducive to fruiting.

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