

limestone ledges or on gravelly soil and can be collected in a nearly pure state.

Colonies of *N. commune* were carefully washed in distilled water by handling with forceps and were then drained free from apparent wetness on filter paper. In this form the weight when dried at 65° C averaged 10% of the drained sample. This figure was used to reduce all determinations to dry weight basis. Portions of 5-g drained weight were allowed to stand in 50 ml of manganese acetate solutions of various concentrations for 3 hr. The solutions were then decanted, and the alga washed 3 times with 50 ml of distilled water. The solutions were at pH 6.7, and these, along with glassware and wash water, were equilibrated to room temperature at 22° C. Manganese adsorbed was determined by analysis of the residual amount in the combined solution and wash water with confirming analyses of the hydrolyzed alga. The result is shown in Fig. 1. It is apparent that adsorption conforms to the Langmuir isotherm.

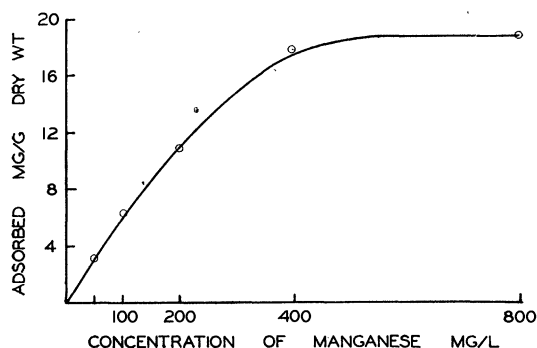


FIG. 1. Manganese adsorbed from manganese acetate solutions by *N. commune*. Ratio of dry wt of alga to volume of solution: 1 g/100 ml.

An analysis of the ashed tunnel deposit made by the Alcoa Reduction Plant Laboratory of the Aluminum Company of America showed Mn(as Mn_2O_3) 50.5%, Fe(as Fe_2O_3) 18.5%, and Ca(as CaO) 1.0%. Since the water passing through the tunnel contained far more calcium and iron than manganese, it seemed desirable to investigate the effect of previous adsorption of these elements on the adsorption of manganese by the alga. *Nostoc*, after washing, contained 9.8 mg/g (dry wt) calcium and 3.5 mg/g iron. Additional calcium was adsorbed from carbonate solutions, but this was loosely held, and the amount retained varied widely with the extent of washing. Iron at 3.5 mg/g was apparently near saturation. A few determinations showed a little was removed from ferric ammonium sulfate and ferric citrate solutions, but the amount could not be accurately determined. Excess iron made the alga so gelatinous that it was impossible to wash it thoroughly.

Experiments on other polysaccharides were run on columns. Berl saddles were coated with melted agar. These were put in a 20- \times 300-mm column, and manganese solutions containing 50 ppm were dripped through at 6 drops/min. Manganese was adsorbed be-

yond the limit of detection. The same result was obtained with starch and with mixed algae from the lake feeding the tunnel.

After adsorption manganese is slowly oxidized, possibly by bacteria, although the slides failed to show any of the often described stalked forms. Only small rod and coccus forms were present in significant numbers.

Although manganese adsorption by polysaccharides has nuisance aspects, it possibly explains the concentration of manganese in residual clay deposits. There are likewise interesting implications concerning the activity of manganese in living organisms.

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The Recovery of Preformed Thrombin from Circulating Plasma

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The absence of thrombin from the general circulation in cases of thrombosis is probably the result of its inactivation by antithrombic substances. The thrombin may, however, be recovered if the thrombin-antithrombin complex is first dissociated with sodium hydroxide (1, 2) and neutralized in the presence of 25% ethanol, which prevents reassociation (3). By these means the theory of thrombin inactivation may be explored and the presence of thrombosis may possibly be determined.

The method for the recovery of thrombin is as follows: Rabbit blood is taken from the median artery of the ear in syringes containing 1 vol 0.1 M sodium oxalate to 9 vol blood, and the plasma obtained by centrifugation. Two and five-tenths ml plasma mixed with 2.5 ml 0.85% sodium chloride solution is brought to pH 11.5 (1° C) with sodium hydroxide dissolved in 25% ethyl alcohol. The reagents are held at 1° C (pH 11.5) for 2 min. Five ml of 50% ethyl alcohol (-2° C) is then added, and the mixture is rapidly brought to pH 7.2 (1° C) with hydrochloric acid dissolved in 25% ethyl alcohol. The recovered suspension of thrombin is brought—after measuring its volume—to 18° C with occasional shaking. Its activity is determined at 18° C by adding 0.2 ml of the suspension to 0.2 ml fresh oxalated rabbit plasma or 0.2 ml 0.3% solution of bovine fibrinogen (Armour) in 0.02 M veronal buffer, pH 7.2, containing 0.16 M sodium chloride solution, and determining the clotting time. The amount of thrombin required to cause clotting of oxalated plasma in 18 sec is designated as one unit. Clotting times above 12 sec are converted into units by the use of a standardization curve. Suspensions giving clotting times below 12 sec are suitably diluted with 25% ethyl alcohol contained in 0.85% sodium

TABLE 1
DEMONSTRATION OF THE RECOVERY OF THROMBIN
ADDED TO AND INACTIVATED BY SERUM

(a) Amount of thrombin added to serum and inactivated by it	72 units
(b) Amount of thrombin recovered from thrombin-serum-plasma mixture	120 "
(c) Amount of thrombin recovered from saline-serum-plasma mixture	45 "
(b) minus (c)	75 units

chloride solution. Determinations are made in triplicate.

Thrombosis was produced in rabbits either by a slight modification of Moses' method (4), in which a strip of wool yarn (about 1 in. long) soaked in rabbit brain thromboplastin is implanted into each external jugular vein under ether anesthesia (Nembutal preparation), or by insertion of silk sutures into both external jugular veins under local novocain anesthesia (Nembutal preparation). Six strips of silk suture were usually implanted along $\frac{3}{4}$ -1 in. of vein. In a third group of animals the external jugular veins were merely exposed. The usual surgical precautions were observed, and the fascia and skin were sutured with plain catgut.

The quantitative nature of the recovery of thrombin was demonstrated by adding 2.0 vol thrombin solution (5) to 0.5 vol serum and incubating the mixture at 18° C until the activity of the added thrombin had completely disappeared. This usually required 45 min. The thrombin recoverable from 2.5 ml of such a thrombin-serum mixture added to 2.5 ml of plasma was compared with the thrombin recoverable from a saline-serum mixture, consisting of 2.0 ml 0.85% sodium chloride solution and 0.5 ml of serum and added to 2.5 ml of the same plasma. The results

(Table 1) show that the difference of the thrombin recovered from these two samples is similar to the quantity of thrombin added to the first sample and inactivated by it.

It was also found that plasma deprothrombinized by absorption with barium sulfate by the method of Rosenfield and Tuft (6) yielded the same amount of recoverable thrombin as unabsorbed plasma. This shows that the thrombin recovered from plasma was actually contained in it as inactive thrombin rather than being formed from prothrombin concurrent with the recovery procedure.

Serum—unlike plasma—did not yield quantitative recoveries of thrombin. Also, plasma defibrinated by the use of thrombin gave low yields, as did serum obtained by recalcification with or without the addition of thromboplastin. We do not know whether this is due to an adsorption of thrombin on the removed fibrin (7) or whether in the absence of fibrinogen part of the dissociated thrombin is denatured upon exposure to alcohol and alkali.

The normal value of recoverable thrombin was determined by individual tests of 35 bleedings from 21 rabbits. The average was 16 units/ml, with a standard deviation of 5.73. Prothrombin determinations (8) of the same plasmas yielded an average of 335 units/ml, with a standard deviation of 42.2.

The effect of experimental thrombosis on the amount of recoverable thrombin is shown in Table 2, which summarizes a few typical pre- and postoperative determinations. In animals Nos. 1-5 the recoverable thrombin levels had been elevated before the post-mortem observation, which revealed fibrosed veins. In the animal dissected on the day the level of thrombin was elevated (No. 9), thrombi were found in both external jugular veins. Animal No. 8 was implanted

TABLE 2
PRE- AND POSTOPERATIVE THROMBIN RECOVERY FROM RABBIT PLASMA

Rabbit No.	Type of operation	Units recoverable thrombin/ml		Autopsy
		Before operation	3rd postoperative day	
1	Wool yarn	16	48	14th postoperative day after return to normal of recoverable thrombin; both external jugular veins fibrosed, lumen obliterated
2	"	14	39	12th postoperative day after return to normal of recoverable thrombin; same finding as in 1
3	"	21	47	Same as 2
4	"	17	31	10th postoperative day after return to normal of recoverable thrombin; same finding as in 1
5	"	13	34	Same as 4
6	Simple exposure	19	33	5th postoperative day after return to normal of recoverable thrombin; no thrombosis or fibrosis
7	Simple exposure	17	23	5th postoperative day while recoverable thrombin was normal; no thrombosis or fibrosis
8	Silk suture	8	23	3rd postoperative day while recoverable thrombin was normal; no thrombosis
9	" "	18	42	3rd postoperative day while recoverable thrombin was elevated; thrombus adherent to vessel wall and to silk thread on both sides, and extending beyond region of silk-thread implantation

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 Radiation¹

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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 con-

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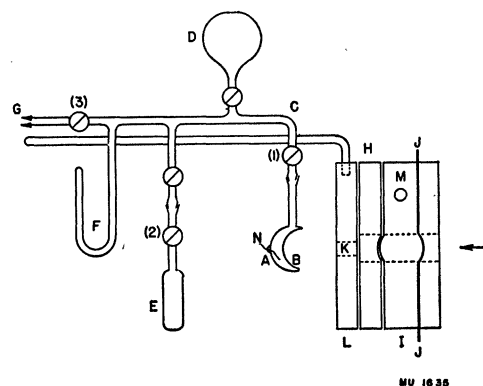


FIG. 1.

ditions 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.

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-