the formation of the corresponding α-keto acids was not reported. Re-examination of the effects of this reagent on various amino acids (e.g., alanine, serine, and leucine) has now shown that α-keto acid formation does take place to some extent. A typical oxidation experiment was conducted as follows:

To a stirred solution of alanine (approx 0.01 moles) were added, dropwise and simultaneously, equimolecular amounts of ferrous sulfate and hydrogen peroxide. The reaction mixture, after acidification and addition of 2:4-dinitrophenylhydrazine solution, was allowed to stand a short while, and the hydrazones were then extracted with chloroform. This extract was washed and shaken with 10% sodium carbonate. The hydrazone of pyruvic acid was precipitated from the carbonate extract on addition of acid—mp, 217°–218° C (after recrystallization from ethanol); no depression of the mp on mixing with an authentic specimen; yield: ∽ 10 mg of pyruvic acid. Better yields may possibly be obtained by changing the experimental conditions.

There are only two other recorded cases of the chemical conversion of alanine to pyruvic acid. Simon and Piaux (*9*) used copper in the presence of oxygen, and Bass (*10*) employed ferrous bicarbonate, also in the presence of oxygen. It is very likely that these oxidations also involve free radicals.

Investigations of the effects of x-rays on aqueous solutions of α-amino acids have shown (*11, 12*) that, *in vacuo,* deamination and decarboxylation occur, with subsequent formation of the corresponding aldehyde (*11*). Some hydroxylamine is also produced under certain conditions (*13*). We have found that irradiation in the presence of oxygen results in the formation of both the α-keto acid and the aldehyde. From irradiated alanine solutions, for instance, pyruvic acid was isolated as the 2:4-dinitrophenylhydrazone. This was identified by its mp and mixed mp and was further characterized by paper chromatography in a butanol-water-NH_3 solvent (*14*).

Table 1 shows some quantitative data obtained at three selected pH values. The keto acid was determined as the 2:4-dinitrophenylhydrazone by the method of Friedemann and Haugen (*15*), and the ammonia by the distillation method of Parnas (*16*).

TABLE 1

IRRADIATION OF ALANINE IN AQUEOUS SOLUTION (0.2%) WITH X-RAYS (200 kv) IN THE PRESENCE OF OXYGEN (Dose = $3 \cdot 6 \times 10^4$ r-units)

pH	Yield in (μ moles/ml) × 10^{-2}	
	Pyruvic acid	Ammonia
2	4.70	7.53
7	4.67	5.73
9	4.50	11.0

It will be noticed that the yield of ammonia depends markedly upon pH, giving a minimum value in the region of the isoelectric point; this is in agreement with previous findings (*11*). Pyruvic acid formation, however, does not depend so strongly on pH.

Irradiation of alanine solutions *in vacuo* yields predominantly acetaldehyde (*11*). This suggests that the oxygen present in the irradiated solution plays a very important part in the mechanism of pyruvate production. It is thus highly probable that pyruvic acid and acetaldehyde are formed through two different intermediates. The mechanism of this reaction is now being studied in detail.

References

1. HABER, F., and WILLSTAETTER, R. *Ber.*, **64**, 2844 (1931).
2. STEIN, G., and WEISS, J. *Nature*, **166**, 1104 (1950).
3. KREBS, H. A. *Biochem. Soc. Symposium*, (1), 2 (1948).
4. HABER, F., and WEISS, J. *Proc. Roy. Soc.* (*London*), A, **147**, 332 (1934).
5. WEISS, J. *Nature*, **153**, 748 (1944); *Trans. Faraday Soc.*, **43**, 314 (1947).
6. DAKIN, H. D. *J. Biol. Chem.*, **1**, 171 (1905–6).
7. NEUBERG, C. *Biochem. Z.*, **20**, 531 (1909).
8. WIELAND, H., and FRANKE, W. *Ann.*, **457**, 1 (1927).
9. SIMON, L. J., and PIAUX, L. *Compt. rend.*, **176**, 1227 (1923).
10. BASS, L. W. *Compt. rend. soc. biol.*, **93**, 570 (1925).
11. STEIN, G., and WEISS, J. *Nature*, **162**, 814 (1948); *J. Chem. Soc.*, 3256 (1949).
12. DALE, W. N., and DAVIS, J. V. *Nature*, **163**, 64, (1949); DALE, W. N., DAVIS, J. V., and GILBERT, C. W. *Biochem. J.*, **45**, 93 (1949).
13. SCHOLES, G., and WEISS, J. *Nature*, **167**, 693 (1951).
14. CAVALLINI, D., FRONTALI, N., and TOSCHI, G. *Ibid.*, **163**, 568 (1949).
15. FRIEDEMANN, T. E., and HAUGEN, G. E. *J. Biol. Chem.*, **147**, 415 (1947).
16. PARNAS, J. K. *Biochem. Z.*, **274**, 158 (1934).

The Adsorption of Manganese by Algal Polysaccharides[1]

Arthur L. Pollard and Peter Byrd Smith

Departments of Chemical Engineering and *Bacteriology, University of Tennessee, Knoxville*

During the course of an investigation into the source of manganese deposits on the walls of a hydraulic tunnel, it was found that the initial layer consisted of partly decomposed mucilaginous polysaccharides. This was determined by installing microscope slides in a by-pass cell and analyzing the deposit by means of the Dreywood anthrone reagent (*1, 2*). After the slides had been exposed for 2 months manganese was readily detected by hydrolyzing the deposit with sulfuric acid and determination by the periodate method (*3*). Small quantities of manganese were detected in the water by the same method.

It was assumed that the polysaccharides came from decomposing algae, since fragments of algae and diatoms were found on the slides along with masses of gelatinous material showing vestiges of structure. This led to a search for an alga in a form suitable to quantitative investigation of the manganese-adsorbing mechanism. Thanks to A. J. Sharp, of the University of Tennessee Botany Department, a source of *Nostoc commune* was found which served the purpose admirably. This organism grows in colonies on bare

[1] This investigation was supported, in part, through a grant from the Aluminum Company of America.

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.

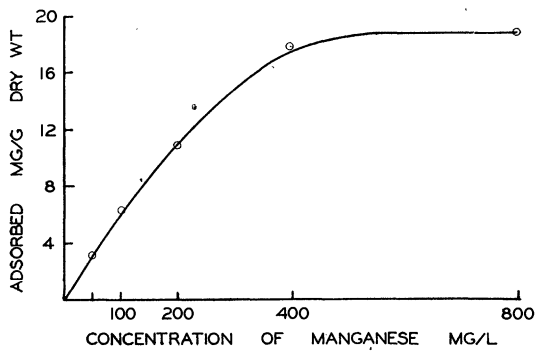

FIG. 1. Manganese adsorbed from manganese acetate solutions by *N. commune*. Ratio of dry wt of alga to volume of solution : 1 g/100 ml.

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- × 300-mm column, and manganese solutions containing 50 ppm were dripped through at 6 drops/min. Manganese was adsorbed beyond 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.

References

1. DREYWOOD, R. *Ind. Eng. Chem., Anal. Ed.,* **18**, 499 (1946).
2. MORRIS, D. L. *Science,* **107**, 254 (1948).
3. RICHARDS, M. B. *Analyst,* **55**, 554 (1930).

The Recovery of Preformed Thrombin from Circulating Plasma

L. A. Sternberger and Frank Maltaner

Division of Laboratories and Research, New York State Department of Health, Albany

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