the (c) curves, which were constant for several minutes) are extrapolated to zero time, values are obtained which differ from each other in agreement with Equation 1.

TABLE V

POTENTIALS OF THE PENTACYANO-NITRITO-FERROATE ELECTRODE

Soln.	N KCl, cc.	N NaNO$_2$, cc.	Ferrous, millimole	Ferric, millimole	E.m.f.	E.m.f. $- E_0'$ Found	E.m.f. $- E_0'$ Calcd.
(a)	50	0	0.15	0.05	0.207[a]	−0.026	−0.028
(b)	50	0	.10	.10	.233[a]	..	..
(c)	50	0	.05	.15	.266	.033	.028
(a′)	50	1	.15	.05	.208[a]	−.027	−.028
(b′)	50	1	.10	.10	.235[a]	..	..
(c′)	50	1	.05	.15	.264	.029	.028
(b″)	50	10	.10	.10	.232[a]	..	..

Mean $E_0' = 0.234$

[a] Extrapolated.

Summary

Under comparable conditions, the oxidation-reduction potentials of the following complex iron cyanides against the normal calomel electrode were: $K_4Fe(CN)_6$–$K_3Fe(CN)_6$, 0.198 volt; $Na_3Fe(CN)_5 \cdot NH_3$–$Na_2Fe(CN)_5 \cdot NH_3$, 0.092 volt; $Na_3Fe(CN)_5 \cdot H_2O$–$Na_2Fe(CN)_5 \cdot H_2O$, 0.209 volt; $Na_4Fe(CN)_5NO_2$–$Na_3Fe(CN)_5NO_2$, 0.234 volt.

Indication was obtained of the formation of the compound, $2Na_3Fe(CN)_5 \cdot H_2O \cdot 3Na_2Fe(CN)_5 \cdot H_2O$.

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[CONTRIBUTION FROM THE MAYO CLINIC]

THE USE OF GELATIN OLEATE MIXTURES FOR THE DEMONSTRATION OF SMALL AMOUNTS OF CALCIUM[1]

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In a study of the penetrability of gelatin by dyes added to blood serum, Bennhold[5] found that it was impossible to use oxalated plasma because the calcium in the gelatin formed a precipitate of calcium oxalate which interfered with the experiments. Although this difficulty can be overcome by the use of ash-free or isoelectric gelatin,[6] it seemed probable that the formation of a stabilized ring reaction might furnish a method for the

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[5] H. Bennhold, "Ueber die Adsorptionsfähigkeit der Serumkolloide tubulär Nierenkranker gegenüber Farbstoffen," *Z. ges. exptl. Med.*, **49**, 71–94 (1928).

[6] Obtained from the Eastman Kodak Company.

detection of minute amounts of calcium or other alkaline earths. It was also decided that results of interest might be obtained by a study of the effect produced by the addition of oxalate, oleate and other anions to a gelatin solution in contact with a solution of the alkaline earths.

Technique

Solutions of ash-free gelatin were so prepared that the final concentration was 5%. These solutions contained substances such as ammonium oxalate, sodium oleate, potassium oleate, sodium fluoride and sodium citrate. Freshly prepared tubes are preferable.

To insure as nearly as possible uniform conditions of temperature the tubes were kept in an ice chest at about 40°. They were read usually after standing from one to four hours, and again on the next day. The tubes, which were 9 mm. in inner diameter, were charged with about 3 cc. of gelatin mixture. The solutions were prepared roughly and the figures are approximate. The solutions to be tested were always added in the amount of 1 cc. The reactions which occurred may be characterized as stabilized ring reactions.

The gelatin in these experiments, as in others in which alkali was added, was dissolved in water, with enough space left in the tube for additions. When dissolved, 20 cc. of 0.01 N solution of oleate for each 100 cc. was added, followed immediately by 12.5 cc. of 0.1 N sodium hydroxide or potassium hydroxide. Then sufficient alkali was added to bring the reaction to a hydrogen-ion concentration of about P_H 7.4, using phenol red as the indicator. The alkalinity varied from about P_H 7.2 to 7.8, but was always greater than P_H 7. In later experiments potassium oleate[7] replaced the sodium oleate. The potassium oleate has the advantage of greater clearness, although both salts behaved similarly.

The water used for the solutions was redistilled in an all-glass apparatus and only ash-free filter paper was used. Tubes with distilled water were used as controls, in none of which was there a reaction. Such controls are necessary for the detection of faint reactions. Reactions which could readily be distinguished from those of the controls were accepted as positive.

The solutions tested were lithium sulfate, sodium chloride, sodium sulfate, sodium nitrate, potassium chloride, potassium iodide, potassium sulfate, magnesium sulfate, strontium chloride, barium acetate, calcium hydroxide, calcium sulfate, calcium acetate, calcium oxalate, sodium hydroxide and potassium hydroxide. These solutions, with the exception of the hydroxides, were brought to a hydrogen-ion concentration of P_H 7.4 to 7.6.

Slight or negative reactions were obtained with the sodium, potassium

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[7] Obtained from Kahlbaum.

and lithium salts in solutions stronger than 0.02 *N*, with reference to the metal; with 0.002 *N* solutions reactions were not obtained. Sodium and potassium hydroxide in concentrations of 0.02 *N* or greater produced some reaction.

The reactions of magnesium solutions were similar to those of the sodium solutions. With concentrations of *M*/400 the reactions were always positive, whereas with concentrations of *M*/4000 they became questionable or negative. Strontium chloride reacted definitely at a concentration of *M*/4000 and questionably at *M*/40,000, whereas barium acetate always reacted at a concentration of *M*/80,000, perhaps even more regularly than the calcium solutions. At a concentration of *M*/160,000 the reaction was questionable or absent.

The calcium compounds, as a rule, also reacted in concentrations of *M*/80,000; occasionally a reaction was questionable or negative. With concentrations of *M*/160,000 a questionable reaction was occasionally seen. Solutions made by exposing freshly precipitated and thoroughly washed calcium oxalate to redistilled water and then brought to a hydrogen-ion concentration of about P_H 7.4 also reacted promptly.

When calcium solutions were added to a watery solution of oleate of the same strength and reaction as in the gelatin tubes, in concentrations lower than *M*/4000, the tubes containing calcium could not be distinguished from the controls. A stratification of the solution could not be produced, as would be expected.

Solutions of gelatin containing 0.001 *N* potassium oleate gave the same results as the usual concentration of 0.002 *N* but with a concentration lower than 0.001 *N* the potassium oleate solutions did not react with the lower concentrations of the calcium solutions.

When ammonium oxalate, sodium fluoride or sodium citrate was added to the gelatin in place of the oleates, it required a greater concentration of calcium solutions to obtain reactions, and in these reactions the precipitate showed peculiar differences in distribution.

Summary and Conclusions

1. Gelatin may be used under certain conditions to obtain ring reactions when a stratification of solutions cannot be accomplished. This method can be amplified.
2. Sodium or potassium oleate mixtures can be used for the detection of small amounts of calcium, the limit being approximately 0.0005 mg. of calcium in 1 cc.
3. The reaction is not specific for calcium.

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