rather than 5 ml., that the Henderson *basal* medium[6] was employed, and that the acid produced by growth of the organisms was titrated to a pH of 6.8 using a Canon titrator[7] and recording the results in counts rather than in milliliters of alkali. In the inhibition tests, 8.0 micrograms of L-lysine was added to the basal medium in each tube and then from 0 to 16 micrograms of ϵ-C-methyllysine were added, testing each amount in triplicate. At the end of the appropriate incubation period, the amount of growth in each tube was determined by titration of the acid produced in the manner described above. Similarly, the ability of L-lysine to overcome the inhibition of the homolog was tested by adding increments of L-lysine to tubes containing, in addition to the basal medium, 8 micrograms of homolog.

Results and Discussion

It should be noted that there are four possible isomers of ϵ-C-methyllysine and that the preparation tested may have contained all of them. Hence the results observed may represent the net effect of a mixture of compounds each of which possesses a characteristic inhibitory (or even stimulatory) influence upon growth. In any case, the preparation, hereafter referred to as the homolog, failed to support growth of *L. mesenteroides* or of *S. faecalis* when amounts of the homolog up to 70 micrograms were substituted for lysine. In parallel tests, definite growth of both organisms was observed with as little as 1 microgram of L-lysine.

Tests were next made to see if the homolog would inhibit growth of the test organisms. Pronounced inhibition of growth was observed as illustrated for *S. faecalis* in curve 2 of Fig. 1. Similar results were obtained with *L. mesenteroides*.[8] In the tubes which received approximately equivalent amounts of lysine and the homolog, and in those which received more homolog than lysine, growth was reduced to about one-quarter of that obtained in the absence of homolog. If molar inhibition ratios[9] are calculated on the basis of the amount of homolog required to reduce the growth level obtained with 8 micrograms of L-lysine to that obtained with one-half that amount, it appears that 1 molecule of homolog can inhibit approximately 3 of L-lysine. However, in view of the finding by Ferger and du Vigneaud[10] that only one of the isomers of β-2-thienylalanine inhibited microbial growth, it is possible that one or more of the isomers of the homolog possesses even greater inhibitory capacity than the preparation tested.

The inhibition of growth of the two test organisms by the homolog could be overcome by addition of L-lysine. This is illustrated for *S. faecalis* in curve 3 of Fig. 1. Inhibition of growth by the homolog was more readily and completely overcome in the case of *L. mesenteroides* than with *S. faecalis*.

Numerous investigations have been made of analogs of metabolites and comparatively fewer tests have been made with homologs. The results of the present tests suggest that it might be profitable to explore further the potentialities of the present and other amino acid homologs in the inhibition of microbial growth.

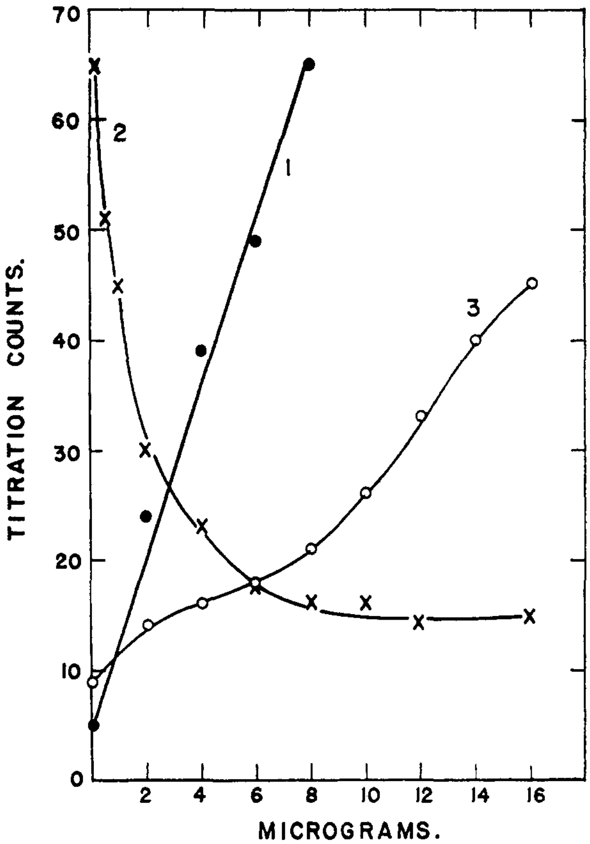

Fig. 1.—Growth of *Streptococcus faecalis* upon addition to basal medium of L-lysine, and various proportions of L-lysine and ϵ-C-methyllysine: curve 1, growth obtained with 0 to 8 micrograms of L-lysine; curve 2, growth obtained with 8 micrograms of L-lysine and 0 to 16 micrograms of ϵ-C-methyllysine; curve 3, growth obtained with 8 micrograms of ϵ-C-methyllysine and 0 to 16 micrograms of L-lysine.

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Millicoulometry. III. The Polarographic Reduction of Copper(II) from Thiocyanate Solutions

BY LOUIS MEITES

The polarographic characteristics of copper(II) in thiocyanate solutions were first studied by Lingane and Kerlinger,[1] who found a double wave, corresponding to stepwise reduction to copper(I) and copper amalgam, in 0.1 F thiocyanate. They reported the half-wave potentials to be −0.02 and −0.39 v., which, although the first is slightly more positive than the depolarization potential of the

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(6) L. M. Henderson and E. E. Snell, *ibid.*, **172**, 15 (1947).

(7) L. M. Henderson, W. L. Brickson and E. E. Snell, *ibid.*, **172**, 31 (1948).

(8) In the case of *S. faecalis*, inhibition of growth in the presence of 8 micrograms of lysine was apparent with 0.5 microgram of homolog or less, whereas inhibition first appeared with *L. mesenteroides* with approximately 4 micrograms of homolog, and up to this level there was definite indication that the homolog was causing an enhancement of growth. However, the ultimate inhibitions at 16 micrograms of homolog were comparable for the two organisms.

(9) K. Dittmer and V. du Vigneaud, *J. Biol. Chem.*, **169**, 63 (1947).

(10) M. F. Ferger and V. du Vigneaud, *ibid.*, **174**, 241 (1948).

(1) J. J. Lingane and H. Kerlinger, *Ind. Eng. Chem., Anal. Ed.*, **13**, 77 (1941).

dropping electrode in this medium, are in line with other data on the copper thiocyanates. More recently it has been stated that in 1.0 F thiocyanate the half-wave potentials of the two one-electron waves are -0.52 and -1.48 v., "in severe disagreement" with the earlier values.[2] It is curious that a typical polarogram shown in support of this claim shows that the "first" wave is much higher than the "second," instead of being equal to it as one would expect. The work here described was designed to test the hypothesis that the "second" wave is actually the "water wave" frequency encountered in solutions of this ionic strength.[3,4]

Polarograms and millicoulometric electrolyses were made with the apparatus and techniques previously described.[5–7] Potassium thiocyanate was purified according to the method of Kolthoff and Lingane.[8] Some polarograms made in a modified conventional H-cell[9] were repeated with a modified Carritt-type cell[9,10] to check on the possibility of an agar effect, but no differences could ever be detected. A few drops of dimethyl phthalate were added to each solution to prevent reduction of the copper(II) by metallic mercury.[11]

Figure 1 shows typical polarograms of copper(II) in thiocyanate solutions of various concentrations. Below 0.2 F thiocyanate these curves are in agreement with those of Lingane and Kerlinger: the half-wave potential of the second wave is -0.42 ± 0.02 v., while that of the first wave, -0.14 ± 0.01 v., is evidently somewhat more negative than the true value because of the superimposed anodic thiocyanate wave. As the thiocyanate concentration is increased, the second wave increases in height at the expense of the first, and above 0.8 F thiocyanate the two waves are completely merged. In 1.0 F thiocyanate $E_{1/2}$ of this wave is -0.62 v., and its height is about 75% of that of the total double wave in 0.05 F thiocyanate, which indicates a decrease of about 45% in the diffusion coefficient of copper(II) over this range. This may be due to a change in the constitution of the predominating complex ion.

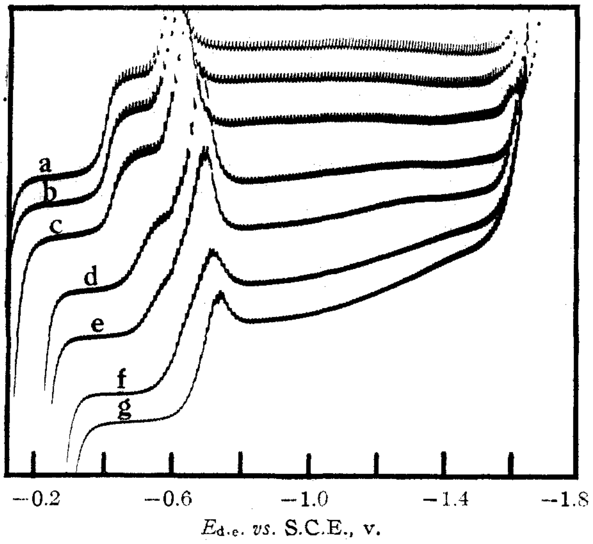

Fig. 1.—Polarograms of *ca.* 1 mM copper(II) in (a) 0.032, (b) 0.062, (c) 0.117, (d) 0.35, (e) 0.57, (f) 0.98 and (g) 1.99 F potassium thiocyanate.

In agreement with Colichman's polarograms, the height of the so-called "second" wave, which appears only at thiocyanate concentrations above 0.5 F, is not equal to that of the wave at -0.62 v., and the ratio of the wave heights depends on ionic strength. Thus I_2/I_1 with 1.0 mM copper is 0.28 in 0.57 F thiocyanate, 0.68 in 2.0 F thiocyanate, and 0.91 in 4.4 F thiocyanate. In 2.0 F thiocyanate this ratio decreases from 1.11 at a copper concentration of 0.09 mM to 0.49 when the copper concentration is 4.3 mM. These observations are fairly conclusive evidence that the "second" wave in concentrated thiocyanate solutions is actually catalytic in nature, and that it cannot be interpreted as being due to the reduction of copper(I).

Further proof of this is afforded by the data presented in Table I. These data show that the reduction corresponding to the first wave in 1.00 F potassium thiocyanate proceeds directly to copper amalgam, and not to a copper(I) complex. Other experiments show that this is also true in solutions having thiocyanate concentrations from 0.85 to 4.4 F.

Since the completion of the work thus far described, the polarography of the copper thiocyanates has been studied by Korshunov and Malyugina,[12] who claimed that in 1-6 F thiocyanate copper(II) is reduced to a cuprous complex (presumably accompanied by the formation of thiocyanogen), and supported this assertion by studies of copper(I) solutions prepared from cuprous chloride.

Solutions containing 1.002 millimoles of copper(II) in 0.5–8.5 F potassium thiocyanate were titrated with a vanadium(II) solution standardized against potassium dichromate.[13] The titrations were made in an atmosphere of nitrogen washed with vanadium(II) perchlorate solutions to remove oxygen.[14,15] The end-point was taken at the disappearance of the yellow or brown color of the copper(II) complex: the change from yellowish-green to clear green is not very sharp, accounting in part for the relatively poor reproducibility of the results. However, the consumption of vanadium(II) at this end-point was in every case $1.00 \pm 0.01_5$ millimole, with no trend with thiocyanate concentrations. This shows unequivocally that copper(II) is not reduced by thiocyanate, for no metallic precipitate could be detected in any solution at the end-point. Excess vanadium(II) did, however, cause a slow precipitation of metallic copper. Except in the most dilute thiocyanate solutions (in which a tur-

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(2) E. L. Colichman, THIS JOURNAL, **72**, 4036 (1950).

(3) I. M. Kolthoff and E. F. Orlemann, *ibid.*, **64**, 833 (1942).

(4) W. Hans and M. v. Stackelberg, *Z. Elektrochem.*, **54**, 65 (1950).

(5) L. Meites and T. Meites, THIS JOURNAL, **72**, 3686 (1950).

(6) S. Bogan, L. Meites, E. Peters and J. M. Sturtevant, *ibid.*, **73**, 1584 (1951).

(7) T. Meites and L. Meites, *Anal. Chem.*, in press.

(8) I. M. Kolthoff and J. J. Lingane, THIS JOURNAL, **57**, 2126 (1935).

(9) L. Meites and T. Meites, *Anal. Chem.*, in press.

(10) D. C. Carritt, Ph.D. Thesis, Harvard University, 1947.

(11) *Radiometer Polarographics*, **1**, no. 2, 6 (1950).

(12) I. A. Korshunov and N. I. Malyugina, *Zhur. Obshchei Khim.*, **20**, 1399 (1950); *C. A.*, **45**, 1456 (1951).

(13) L. Meites, *J. Chem. Education*, **27**, 458 (1950).

(14) L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948).

(15) Vanadium(II) solutions in sulfuric acid liberate traces of hydrogen sulfide after long standing.

bidity developed during the titration) the solutions at the end-point were always perfectly clear, and remained so on boiling. After considerable dilution, however, white precipitates of copper(I) thiocyanate formed: such precipitates could not be secured from copper(II) thiocyanate solutions even after long standing.

TABLE I

MILLICOULOMETRIC ELECTROLYSIS OF COPPER(II) IN 1 *F* THIOCYANATE

The solution initially contained 0.500 micromole of copper(II) in 0.497 ml. of 1.00 *F* potassium thiocyanate. $E_{\text{d.e.}} = -0.90$ v. The blank counting rate of the millicoulometer was 0.2960 ± 0.0001 count/sec., including the contribution from the residual current, and the sensitivity was $4.53_7 \times 10^{-4}$ microfaraday/count. Galvanometer deflections have been corrected for the residual current.

Time	Register	Counts	Counts, cor.	Microfaraday	*d*, cm.	Micromole reduced	*n*
0	11745	0	0	0	30.27	0	..
1000	12178	433	137	0.062	28.40	0.0312	1.99
2000	12602	857	265	.120	26.69	.0593	2.02
3000	13019	1274	386	.175	25.08	.0859	2.04
4000	13430	1685	501	.227	23.56	.111	2.05
5000	13834	2089	609	.276	21.80	.140	1.97
8000	14993	3248	880	.399	18.01	.203	1.97
12000	16472	4727	1175	.533	14.36	.263	2.03
						Mean:	2.01 ± 0.03

As it is not uncommon to find reducing impurities in ammonium thiocyanate preparations, it seems probable that the copper(II) in the solutions of Korshunov and Malyugina was reduced to the +1 state by some adventitious contaminant.

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Observations on the Rare Earths. LXI. Precipitation of Hydrous Oxides or Hydroxides from Perchlorate Solutions[1]

BY THERALD MOELLER AND NORMAN FOGEL

The utilities of data obtained from electrometric titrations of salt solutions with alkalies in establishing the relative basicities of the tripositive rare earth metal ions are well known.[2,3] Both precipitation *p*H values and solubility product constants calculated for the hydrous hydroxides indicate basicity decreases paralleling decreases in ionic radii, but there are sufficient differences among data for nitrate, chloride, sulfate and acetate solutions of comparable concentrations[3] to suggest that cation–anion interactions are of some importance. Although such interactions would probably be of least consequence in nitrate solutions, it was deemed advisable to carry out parallel studies on solutions containing the non-complexing perchlorate ion.

Experimental

Materials Used.—The majority of the rare earths employed were either the same as those used previously[2] or of the same degrees of purity. With praseodymium (PR-43, 99% Pr_6O_{11}), europium (EU-5, 92.7% Eu_2O_3, 6.7% Sm_2O_3), and erbium (ER-1-2s, 98% Er_2O_3, 2% Y_2O_3), materials of slightly different compositions were used. Approximately 0.1 *M* (0.02 *M* with europium) perchlorate solutions were prepared by treating slight excesses of the oxides with 72% perchloric acid, filtering, and diluting to volume. Such solutions were standardized by the usual oxalate–oxide procedure. Carbonate-free sodium hydroxide solutions were standardized against potassium hydrogen phthalate.

Titration Procedure.—Titrations were carried out at 25 ± 0.5° exactly as previously described.[2] All solutions were stirred with nitrogen to minimize carbon dioxide interference. Measurements of *p*H were made immediately after each addition of alkali and at three-minute intervals until constant values were obtained.

Results

Numerical data characterizing these titrations are summarized in Table I. It is apparent that data for perchlorate solutions are closely comparable with those already reported[2] for nitrate solutions. The differences in basicity between ions of adjacent elements are somewhat better defined, and the well-known excessively high basicity of the lanthanum ion is somewhat more apparent for perchlorate solutions. In addition the precipitation ranges for all of the cations were found to embrace smaller *p*H intervals than with the nitrate solutions. The apparently anomalous behavior of praseodymium shown in previous studies[2] was also resolved, and the titration curve for praseodymium perchlorate lay close to and above that for the neodymium salt and uniformly considerably below that for the lanthanum salt. Use of a more nearly pure praseodymium sample doubtless accounts for the difference.

TABLE I

ELECTROMETRIC TITRATION DATA FOR PERCHLORATE SOLUTIONS

Ion	Precipitation *p*H At precipitation incidence	At $OH^-/R^{+++} = 0.4$	Average solubility product constant	Average water solubility ($\times 10^6$) mole/liter
La^{+++}	8.10	8.18	1.7×10^{-19}	8.8
Pr^{+++}	7.40	7.43	6.7×10^{-22}	2.2
Nd^{+++}	7.30	7.34	3.2×10^{-22}	1.9
Sm^{+++}	7.13	7.17	8.4×10^{-23}	1.3
Eu^{+++}	6.91	6.99	0.9×10^{-23}	0.8
Gd^{+++}	6.84	6.90	1.8[illegible] $\times 10^{-23}$	.9
Y^{+++}	6.81	6.88	1.6×10^{-23}	.9
Er^{+++}	6.61	6.68	4.1×10^{-24}	.6
Yb^{+++}	6.45	6.55	2.5×10^{-24}	.5
Lu^{+++}	6.45	6.55	1.9×10^{-24}	.5

Titration data for perchlorate solutions support the theoretical basicity order for these cations and place yttrium in its logical place between gadolinium and erbium. That precipitation was complete in all cases at $OH^-:R^{+++}$ mole ratios of 2.60–2.75 suggests basic salt formation to be at a minimum in perchlorate solutions.

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[1] For the preceding communication in this series, see T. Moeller and F. A. J. Moss, THIS JOURNAL, **73**, 3149 (1951).

[2] T. Moeller and H. E. Kremers, *J. Phys. Chem.*, **48**, 395 (1944).

[3] T. Moeller and H. E. Kremers, *Chem. Revs.*, **37**, 97 (1945).