be fruitful. On the basis of the results presented here and some of those presented elsewhere, especially by Lundegårdh (*10*), malate (or maleic acid) does not appear to be a plant growth inhibitor, but may actually be utilized by at least some plants. However, in view of the inhibitory effect of maleic acid on animal respiration and mitosis, the possibility that under certain conditions it may act in a similar capacity in plants cannot be discarded. The rather conflicting results with maleic acid could possibly be due to its enzymatic conversion into related naturally occurring metabolic acids in at least some plants under certain conditions.

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The Reduction of Vitamin B_{12}

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We have carried out a titrimetric reduction of cyanocobalamin with chromium (II) ethylenediamine tetraacetate complex in a specific application of a new general analytical technique to be described in detail elsewhere. Here we shall list some observations concerning the nature of reduced vitamin B_{12} which are at variance with those reported by Diehl *et al.* (*1, 2*).

In 0.1 *M* sodium enta, pH 9.5, cyanocobalamin gives a polarogram comprising a single reductive wave, $E_{1/2} = -1.021$ v vs saturated calomel electrode (25°). When this solution is titrated amperometrically with standard 0.1 *N* chromium (II) chloride under rigorous exclusion of oxygen, the diffusion current corresponding to the above wave diminishes linearly with volume of titrant; at the same time an *anodic* wave, $E_{1/2} = -0.311$ v vs saturated calomel electrode appears whose diffusion current reaches maximum development

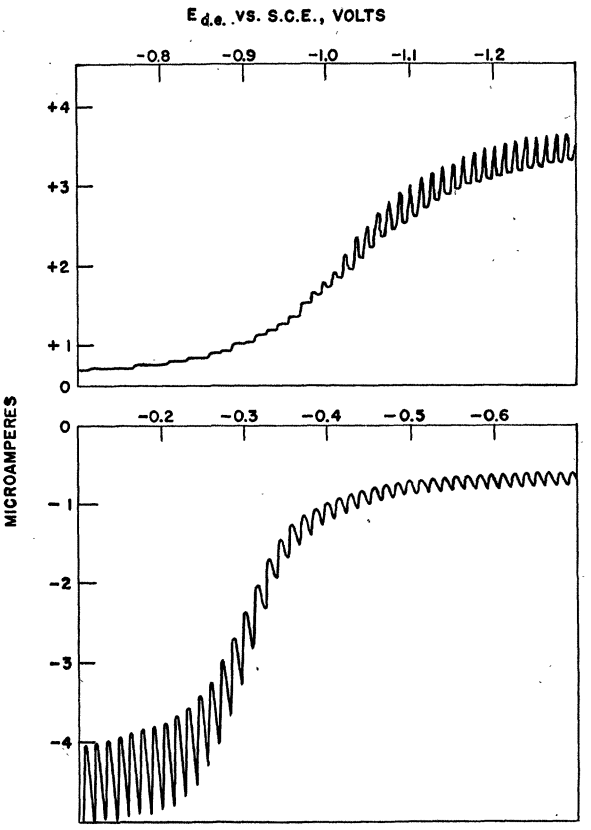

FIG. 1. Top, cathodic wave of vitamin B_{12}; bottom, anodic wave of reduced vitamin B_{12}.

coincident with the vanishment of the cyanocobalamin wave. During this operation the color of the solution changes from red to brown. Polarograms of cyanocobalamin and its reduction product are shown in Fig. 1; titrimetric data on replicate determinations are given in Table 1, and polarographic data in Table 2.

It is evident that cyanocobalamin and its reduction

TABLE 1*

Cyanocobalamin (mg)	Milliequivalents of Cr^{++}	Equivalent weight of cyanocobalamin
13.62	0.01017	1341
2.224	0.001679	1342

* The sample of cyanocobalamin used in this investigation was 99.8% pure by solubility analysis on dry basis; drying loss was 22.8%. All data were obtained on hydrous crystals and corrected accordingly.

TABLE 2

		Cyanocobalamin	Reduction product
$E_{1/2}$ v vs S.C.E.		−1.021 (1.362 mg/cc)	−0.311 (0.3094 mg/cc)
$I_d/Cm^{2/3}t^{1/6}$	(C in mg/cc)	0.219	0.318

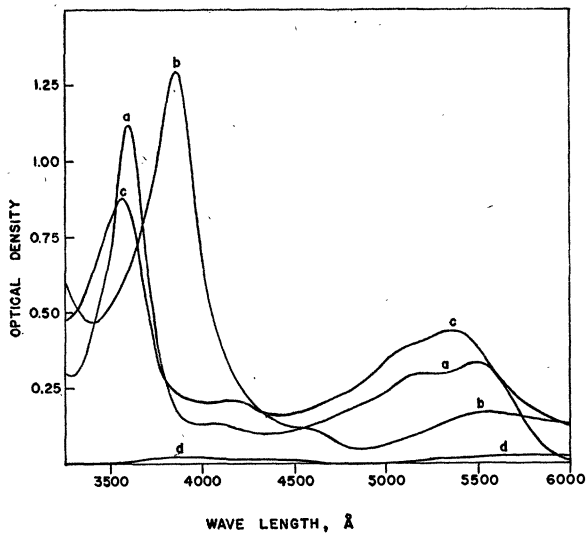

FIG. 2. Absorption spectra: *a*, vitamin B_{12}; *b*, reduced vitamin B_{12}; *c*, air-oxidized product; *d*, (Cr enta)$^-$.

product form a system irreversible at the dropping mercury electrode, a situation not unexpected in view of the known loss of cyanide from cyanobalamin on reduction; furthermore, the equivalent weight of cyanocobalamin found by reductive titration corresponds to a 1-electron transfer. The brown reduction product is not oxidized by bromate ion, whereas (Cr enta)$^=$ is quantitatively oxidized; however, exposure to oxygen at once eliminates the anodic wave, and the solution turns red. Interestingly, when the air-oxidized material is back-titrated with chromium (II) complex, the solution becomes brown again, but no point can be reached at which an excess of (Cr enta)$^=$ is present; apparently the product catalyzes the reduction of water by Cr^{++}.

Diehl (*1, 2*) presents a polarogram obtained on a product termed by him vitamin B_{12r}, produced by reduction of cyanocobalamin with hydrogen on platinum catalyst. Two waves are shown at $E_{1/2} - 0.75$ and -1.37 v vs saturated calomel electrode; although their nature is not specifically defined, they are presumed to be cathodic, by comparison with a polarogram of cyanocobalamin superposed on the graph. This is surprising, because it would be anticipated that a substance having the oxygen avidity of reduced vitamin B_{12} would show anodic depolarization properties, as we indeed found.

The absorption spectra of reduced cyanocobalamin, its air oxidation product, and (Cr enta)$^-$ ion at the end point of the titration are illustrated in Fig. 2, with numerical data in Table 3. Again, our reduction product differs radically from that of Diehl, for which absorption maxima are listed at 4730, 4050, and 3125 Å. Since the contribution to the absorption spectrum of reduced cyanocobalamin solution by (Cr enta)$^-$ is evidently negligible, the differences are real.

TABLE 3

ABSORPTION MAXIMA OF REDUCED VITAMIN B_{12}

Wavelength (Å)	$E^{1\,cm}_{1\%}$
3853	179
4597	12.5
5537	23.6

Since Diehl reports that his vitamin B_{12r} could be back-titrated with potassium ferricyanide (consuming exactly 1 equivalent), the question is raised whether more than one reduction product of cyanocobalamin is possible. Inspection of Diehl's polarographic data yields some pertinent information. He concludes that the polarographic reduction of cyanocobalamin involves two electrons ($Co^{3+} \rightarrow Co^{+}$) and that of his vitamin B_{12r}, two stages of 1 electron each ($Co^{2+} \rightarrow Co^{+} \rightarrow Co^{0}$). However, his polarograms of cyanocobalamin and vitamin B_{12r} cover almost exactly the same voltage range, a situation difficult to reconcile with the different final valence states of cobalt which are assumed. We have obtained polarograms on various noncrystalline degradation products of vitamin B_{12} which closely resemble that presented by Diehl for vitamin B_{12r}; in this connection, we are informed by E. A. Kaczka that the maximum yield of catalytic reduction products of cyanocobalamin which are regenerable to the starting material approximates 70%. Hence the conclusions of Diehl concerning the identity of his vitamin B_{12r} do not appear justifiable on the basis of evidence which he presents. For the polarographic reduction of cyanocobalamin to a compound of univalent cobalt, no such assessment is possible unless it can be shown that reduction of the organic part of the molecule cannot occur.

In conclusion, the evidence at our disposal indicates that the valence states of cobalt in vitamin B_{12} compounds are the normal 2 and 3, and that in the divalent state the anticipated anodic depolarization properties are present.

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Effect of Toluidine Blue on the Coagulation of Fibrinogen by Thrombin[1]

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Haley and Stolarsky (*1*) pointed out that, although Toluidine Blue was capable of inactivating heparin and decreasing coagulation time *in vitro*, the range

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