and

$$\frac{w(0,0) - w(E,\psi_0)}{kT} = M \ln \frac{\xi(\lambda)\xi^0(\lambda')}{\xi^0(\lambda)\xi(\lambda')} - \frac{M}{24}\left(\frac{lqE}{kT}\right)^2 \{[\lambda'] - [\lambda']^0\} \quad \text{(III.3)}$$

That is, even when $E = 0$ there is an effect, if $\psi_0 \neq 0$, arising from the fact that the number of ions bound depends on ψ_0.

Section III.—We replace λ by λ' in eq. 18. Equations 21–23 are all unaffected except for replacement of λ by λ'. We note, incidentally, that

$$kT\left(\frac{\partial \ln Y}{\partial \psi_0}\right)_{T,B\alpha,B,\mu,E} =$$

$$kT\left(\frac{\partial \ln Y}{\partial \lambda}\right)_{T,B\alpha,B,E}\left(\frac{\partial \lambda'}{\partial \psi_0}\right)_{\lambda,T}\left(\frac{\partial \lambda}{\partial \lambda'}\right)_{\psi_0 T} =$$

$$kT \times \frac{\overline{N}}{\lambda} \times -\frac{\lambda' q}{kT} \times \frac{\lambda}{\lambda'} = -\overline{N}q$$

as would be expected on thermodynamic grounds. A similar result follows above (for Section II).

EUGENE, OREGON

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Reversible Uptake of Oxygen by Vitamin B_{12a}

BY BRUNO JASELSKIS AND HARVEY DIEHL

RECEIVED JUNE 20, 1957

The apparent specific volumes of vitamins B_{12} and B_{12a} have been measured, the values obtained being: for B_{12} 0.665 (independent of the presence or absence of oxygen); for B_{12a} 0.650 (in the absence of oxygen) and 0.713 (in the presence of oxygen). An amperometric titration of B_{12a} with a standard solution of oxygen confirmed the earlier finding that vitamin B_{12a} when placed in solution dimerizes through the agency of oxygen. It was further established that the combining ratio is two molecules of B_{12a} to one molecule of oxygen. The amperometric titration method further showed that vitamin B_{12} does not combine with oxygen. This is also in agreement with the earlier measurements of diffusion coefficients and apparent specific volumes. An amperometric titration of vitamin B_{12r} with oxygen gave results in accord with the concept that B_{12r} is a bivalent cobalt compound which is easily oxidized to B_{12a}. The titration showed two end-points corresponding first to the oxidation of the cobalt and second to the dimerization of B_{12a}. Vitamin B_{12a} combines reversibly with oxygen gas, this being the first case of a trivalent cobalt compound to exhibit such behavior.

A repetition[1] of the measurements of the diffusion coefficients of vitamins B_{12} and B_{12a} confirmed the earlier report[2] that the molecular weight of vitamin B_{12a} in solution is twice that of B_{12}. The newer measurements of the diffusion coefficients were made by a free diffusion method using the Tiselius electrophoresis apparatus (without applied potential); the results probably are accurate to within 5%. The molecular weights were calculated by both the Stokes–Einstein and the Stokes–Einstein–Longsworth[3] equations, the latter giving values for the molecular weight of B_{12} in reasonable agreement with that calculated on the cobalt content. Calculated by either method, however, the molecular weight of B_{12a} appeared to be twice that of B_{12}. Moreover, these results were confirmed by a measurement of the sedimentation coefficients and of the apparent specific volumes; these values together with the diffusion coefficients make possible a calculation of molecular weight by the Svedberg equation.

These studies showed in a gross way that vitamin B_{12a} dimerizes in water solution but offered no mechanism by which the dimerization might occur. A clue to this was obtained during the course of the density measurements. Erratic results were obtained in the initial measurements on B_{12a} although no difficulty was experienced with B_{12}. The variation was traced to the time of contact of the solutions with the atmosphere and ultimately to oxygen. That oxygen and vitamin B_{12a} do interact was shown then by density measurements and by amperometric titrations of B_{12a} with oxygen. The combining ratio is two molecules of B_{12a} to one molecule of oxygen. This is apparently the first record of a trivalent cobalt compound combining reversibly with molecular oxygen.

A. Apparent Specific Volumes of B_{12} and B_{12a}

The density measurements were made by the pycnometer method and the calculations made using the usual relationship

$$v_s = \frac{1}{\rho_0} - \left(\frac{\rho_0 - \rho_s}{\rho_0}\right)\left(\frac{V}{g}\right)$$

in which v_s is the apparent specific volume, ρ_0 and ρ_s the densities of water and solution, respectively, V the volume of the pycnometer, and g the weight of the solute.

Materials.—Vitamin B_{12}, obtained from the Squibb Institute for Medical Research, New Brunswick, N. J., was recrystallized from carbon dioxide-free water. Oxygen-free nitrogen was prepared by passing tank nitrogen through two scrubbers of vanadous sulfate, one scrubber of sodium hydroxide, and one of water.

Vitamin B_{12a} was prepared from crystalline vitamin B_{12} by the hydrogenation procedure.[4]

Apparatus and Procedure.—A 5.0-ml. pycnometer was used. Weighings were made using tares of identical weight and volume. Solutions were kept in a water-bath at 25.00 ± 0.01°. The balance room was maintained at slightly below 25°.

The pycnometer was charged with liquid already brought to equilibrium with oxygen-free nitrogen, air or oxygen. Water was placed in a small conical flask bearing a two-holed rubber stopper carrying lengths of glass tubing one of which reached the bottom of the flask and the second of which served as a gas outlet. The gas was bubbled through the solution for several minutes. The crystalline vitamin was added through the gas outlet tube and thus dissolved in the water without the stopper having been removed. The gas stream was then continued an additional 30 minutes. The solution was then transferred to the pycnometer with a hypodermic syringe. In the oxygen-free experiments the pycnometer and syringe were well flushed with nitrogen and

(1) B. Jaselskis, J. F. Foster and H. Diehl, *Iowa State Coll. J. Sci.*, **31**, 1 (1956).

(2) H. Diehl, R. R. Sealock and J. Morrison, *ibid.*, **24**, 433 (1950).

(3) L. G. Longsworth, THIS JOURNAL, **75**, 5705 (1953).

(4) E. Kaczka, D. E. Wolf and K. Folkers, *ibid.*, **71**, 1514 (1949).

the operations were carried out in a large beaker filled with continuously flowing nitrogen gas. The pycnometer was then kept in the water-bath for 45 minutes. After the pycnometer was taken out of the bath it was rinsed with alcohol and dried with a moist chamois skin and handled without contact with the hands. Weighings were made after 10 minutes, with the usual precautions to minimize static electrical effects.

After weighing, an aliquot of 0.200 ml. was taken for cobalt analysis.

The maximum error in this determination of apparent specific volume is estimated to be less than 3% on the basis that the errors in the individual measurements were not greater than 0.01° in temperature, 0.00006 ml. per ml. in the volume of pycnometer, 0.00002 g. in the individual weighings, and 0.60% of vitamin as obtained from the cobalt determination.

The effect of dissolved air on the density of water is noticeable only in the sixth place.

Results and Discussion.—The results of the various measurements of the apparent specific volumes of B_{12} and B_{12a} are presented in Table I. The average values obtained were

B_{12}, (runs 1, 2, 3 and 4)	0.665
B_{12a}, deaerated (runs 10, 12 and 13)	.650
B_{12a}, in contact with oxygen (runs 5, 6, 7, 9, 11, 14 and 15)	.713

The apparent specific volume of B_{12} is independent of the gas with which the solution is equilibrated as shown by experiments 1, 2, 3 and 4, Table I. The apparent specific volume of B_{12a}, however, is dependent upon the presence of oxygen, 0.713 in the presence of oxygen, 0.650 in its absence. A 15-minute period sweeping with nitrogen gas proved to be insufficient time for complete removal of the oxygen (expt. 8, Table I) but a period of 40 minutes sufficed.

The densities of solutions are linear with concentration as expected, as shown in Fig. 1.

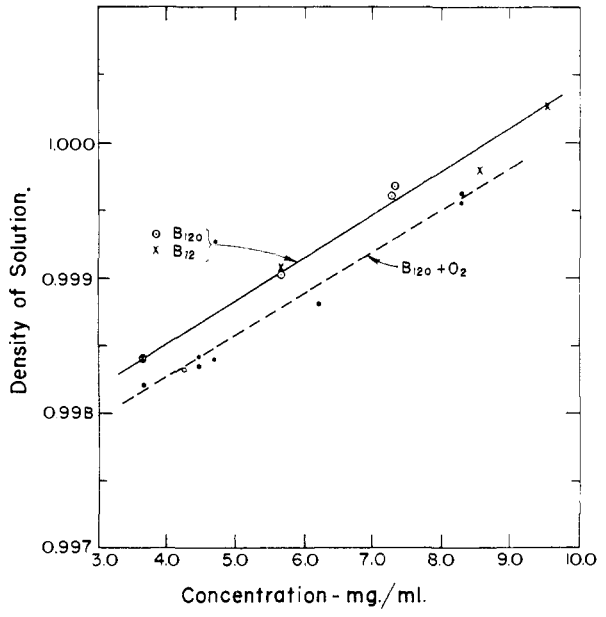

Fig. 1.—Density as a function of concentration.

B. Amperometric Titration of B_{12a}, B_{12} and B_{12r} with Oxygen

Given the information that oxygen is involved in the dimerization of vitamin B_{12a} in aqueous solution, we considered the various ways in which the stoichiometry of the dimerization reactions could be investigated and finally settled on the polarographic method as being possible with the small amounts of material available.

TABLE I

APPARENT SPECIFIC VOLUME OF VITAMINS B_{12} AND B_{12a}

Expt. no.[a]	Material	Equilibrating gas	Density of soln.	Wt. of vitamin, mg./ml.	Vol. ml./g.
1-1	B_{12}	Air	1.00031	9.557	0.662
2-2	B_{12}	Air	0.99978	8.545	.670
3-3	B_{12}	Nitrogen	.99903	5.751	.662
4-3	B_{12}	Oxygen	.99907	5.751	.669
5-5	B_{12a}	Air	.99876	6.127	.727
6-6	B_{12a}	Air	.99840	4.648	.715
7-7	B_{12a}	Oxygen	.99834	4.545	.720
8-7[b]	B_{12a}	Nitrogen	.99842	4.545	.706
9-9	B_{12a}	Air	.99832	4.325	.716
10-9(10)[c]	B_{12a}	Nitrogen	.99844	3.775	.639
11-10	B_{12a}	Oxygen	.99821	3.775	.703
12-12[d]	B_{12a}	Nitrogen	.99969	7.379	.651
13-12[e]	B_{12a}	Nitrogen	.99961	7.379	.660
14-14[f]	B_{12a}	Oxygen	.99965	8.425	.707
15-14[g]	B_{12a}	Oxygen	.99956	8.425	.702

[a] The second number indicates which solution was used for the measurement; thus runs 3 and 4 were made on the same solution. [b] In run 8, nitrogen was bubbled through the solution for 15 minutes only; the oxygen was apparently incompletely removed in this time. [c] In run 10, nitrogen was bubbled through solution 9 for 14 hours; the resulting solution was given the new number 10 because of the dilution on transfer. [d] In run 12, the water was thoroughly deaerated before the B_{12}t was dissolved. [e] Solution 12 was allowed to stand for 14 hours and the measurements were repeated. [f] In run 14, oxygen was bubbled through a fresh solution for four hours. [g] Oxygen was bubbled through the solution overnight.

The polarography of vitamins B_{12} and of B_{12a} has already been investigated.[5-7] B_{12} shows a single two-electron reduction at a half-wave potential of −1.12 v. toward the S.C.E. Vitamin B_{12a} shows two one-electron reduction waves at half-wave potentials of −0.04 and −1.02 v. toward the S.C.E. Oxygen dissolved in water shows two two-electron reduction waves, half-wave potentials −0.08 and −0.96 v. toward the S.C.E., corresponding, respectively, to the reduction of oxygen to hydrogen peroxide and of the latter to water. If B_{12a} and oxygen were present in the same solution and if no interaction were to occur, the wave heights of the first reduction waves would be simply additive inasmuch as the half-wave potentials of the first reduction waves of the two substances are practically the same. On the other hand, if interaction occurs then a shift should be observed in the half wave potential and the wave heights should not be additive.

In order to vary the concentration of one component of the mixture, we delivered the oxygen as a saturated solution in the supporting electrolyte, in effect converting the study to an amperometric titration. Similar amperometric titrations were carried out with solutions of B_{12} and of B_{12r}.

(5) H. Diehl, R. R. Sealock and J. Morrison, *Iowa State Coll. J. Sci.*, **24**, 433 (1950).

(6) H. Diehl, J. I. Morrison and R. R. Sealock, *Experientia*, **7**, 60 (1951).

(7) B. Jaselskis and H. Diehl, THIS JOURNAL, **76**, 4345 (1954).

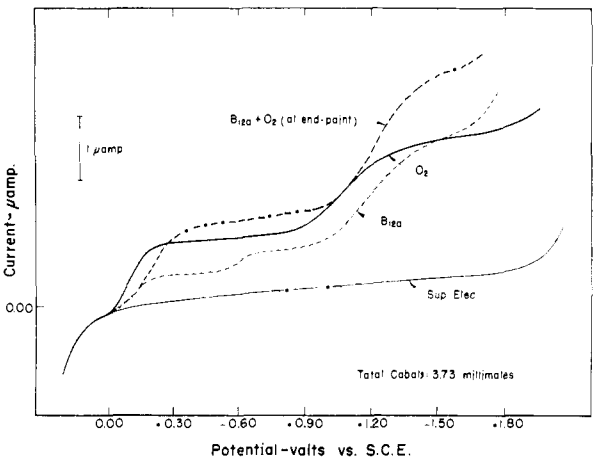

Fig. 2.—Polarograms of vitamin B_{12a}, oxygen, B_{12a} plus oxygen (at end-point), and supporting electrolyte. Curves for B_{12a} and B_{12a} plus oxygen were obtained on the same solution; that of oxygen was obtained on a solution containing less oxygen than required to reach the end-point.

Materials and Reagents.—Vitamin B_{12}, obtained from the Squibb Institute for Medical Research, New Brunswick, N. J., was recrystallized from deionized water. Vitamin B_{12a} was prepared as described above.

Oxygen-free nitrogen was prepared as described in the preceding section. The gas was led to the apparatus through all glass tubing.

Electrolytic hydrogen was obtained from the low temperature laboratory of the Department of Physics, Iowa State College. The impurities in the hydrogen were determined by Dr. Harry J. Svec using the mass spectrograph. Oxygen, carbon dioxide, methane and other gaseous impurities commonly found in commercial cylinder hydrogen were absent.

Potassium sulfate, used for the preparation of supporting electrolytes solutions, was recrystallized from deionized water.

Cylinder oxygen was passed through a tower of ascarite to remove acidic gases present.

A standard solution of oxygen was prepared by bubbling purified oxygen gas through 0.1 *N* potassium sulfate solution for 6 hours. The solution was stored in a Machlett buret in which pure oxygen was maintained slightly above atmospheric pressure by a balloon inflated with oxygen and attached to the upper side arm of the buret.

Apparatus.—A Sargent Model XXI polarograph was used. The functional operation of this instrument was checked frequently against a standard resistance. The polarograph cell used was the usual type so arranged that the tip of the Machlett buret containing the standard oxygen solution was brought in parallel to the capillary and to the salt bridge of the saturated calomel electrode.

A Beckman DU spectrophotometer was used in making the colorimetric determinations of cobalt.

Determination of the Oxygen in the Standard Solution.—In preliminary work the concentration of the oxygen in the standard solution was obtained by (a) interpolating the values for the solubility of oxygen at various temperatures as given in Lange's Handbook and (b) by calculation from polarographic data. In later work, the oxygen concentration was determined by direct chemical measurement, either by the chromous chloride method[8] or by the Winkler method.[9] The agreement between the various methods was quite satisfactory.

Determination of Cobalt.—Aliquots of various solutions were analyzed for cobalt by first destroying the organic matter by fuming with perchloric acid and then determining the cobalt with 2-nitroso-1-naphthol-4-sulfonic acid.[10]

(8) H. W. Stone and R. L. Eichelberger, *Anal. Chem.*, **23**, 868 (1951).

(9) American Public Health Association, "Standard Methods for the Examination of Water and Sewage," 8th Ed., 1936, pp. 139–154.

(10) W. M. Wise and W. W. Brandt, *Anal. Chem.*, **26**, 693 (1954).

Amperometric Titration of B_{12a}.—Exactly 5.00 ml. of the supporting electrolyte and 0.1 *N* potassium sulfate, were placed in the polarograph cell. A platinum boat containing the crystalline B_{12a} was hung above the liquid in the cell. The cell was flushed thoroughly with oxygen-free nitrogen. Boat and B_{12a} were then dropped into the solution. After sufficient time for dissolution and mixing, the polarogram was recorded. A small portion of the standard oxygen solution now was added, the solution was stirred gently, and the polarogram was then recorded. A faint stream of nitrogen was passed over the surface of the liquid during these operations. A further volume of the standard oxygen solution next was added and the polarogram again recorded. This sequence was repeated until sufficient oxygen had been added to have combined with the B_{12a}. At the end of the titration the solution was diluted with a measured amount of the standard oxygen solution to exactly 10.0 ml. and an aliquot of 0.200 ml. was taken for a determination of cobalt. The temperature throughout the titration was maintained at 25 ± 0.2°.

The diffusion current was measured on the recorded polarograms by the standard procedure. Using the values for the diffusion currents obtained experimentally, values were calculated for the diffusion current which would have been obtained had there been no dilution

$$i_d, \text{ cor.} = i_d \text{ meas.} (V + v)/V$$

in which V is the initial volume of the supporting electrolyte, and v the volume of the titrant added.

Typical polarograms are shown in Fig. 2. The results of a representative titration are summarized in Table II and shown graphically in Fig. 3. The end-point for this titration was found to be 2.18 ml.

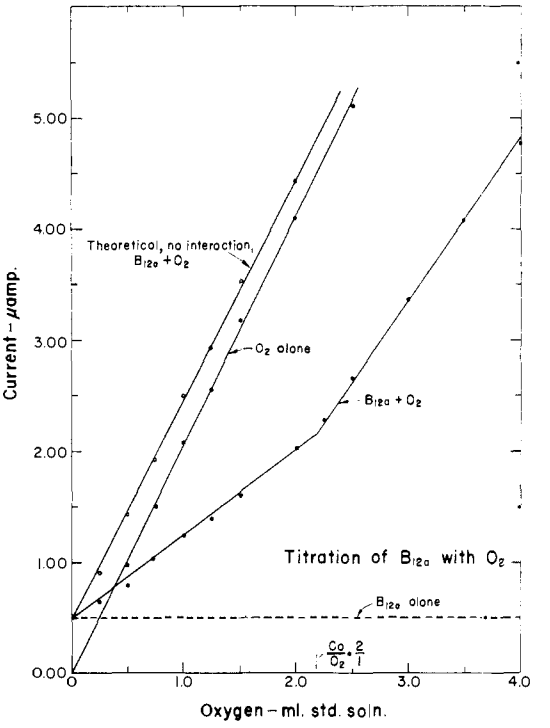

Fig. 3.—Amperometric titration of B_{12a} with standard oxygen solution.

Amperometric Titration of B_{12r}.—Crystalline B_{12} was dissolved in about 5.2 ml. of 0.1 *N* potassium sulfate, and it was hydrogenated for 7 hours using platinum as a catalyst. The hydrogenated solution was transferred from the hydrogenation vessel to the polarograph cell through a fritted glass filter, all joints in the apparatus being of ground glass. The cell and all the apparatus were continuously flushed with a stream of nitrogen and hydrogen. Nitrogen was bubbled for additional 5 minutes through the solution and then the polarogram was recorded. A small portion of the standard oxygen solution was then added, the solution was stirred gently, and the polarogram was recorded. This sequence

TABLE II

POLAROGRAPHIC BEHAVIOR OF SOLUTIONS OF OXYGEN AND OF B_{12a} PLUS OXYGEN

Initial volume of the supporting electrolyte, 5.00 ml.; initial concentration of B_{12a}, 0.746 mmolar.

Oxygen added Vol. of standard soln.,[a] ml.	Quantity, mmoles	Diffusion current obsd., μamp. Oxygen alone[b]	B_{12a} plus oxygen	Diffusion current calcd. to initial vol. of 5.00 ml. μamp. Oxygen alone	B_{12a} plus oxygen[c] assuming no interaction	B_{12a} plus oxygen obsd.[d]
0.00	0.000	0.00	0.51	0.00	0.51	0.51
.25	.214	.43	.60	.45	0.96	.63
.50	.427	.88	.70	.97	1.48	.77
.75	.641	1.30	.96	1.50	2.01	1.10
1.00	.855	1.75	1.07	2.10	2.61	1.28
1.25	1.069	2.05	1.12	2.56	3.07	1.40
1.50	1.283	2.45	1.24	3.18	3.69	1.61
2.00	1.710	2.92	1.46	4.09	4.60	2.04
2.25	1.924	3.14	1.61	4.55	5.06	2.34
2.50	2.137	3.36	1.77	5.04	5.55	2.65
3.00	2.565	3.80	2.10	6.08	6.59	3.36
3.50	2.993	4.12	2.39	6.80	7.31	4.06
4.00	3.420	4.53	2.74	8.05	8.56	4.93

[a] Concentration of the standard solution: (1) 26.9 p.p.m. (from the polarographic data and calculations using Ilkovic equation); (2) 27.4 p.p.m. (Winkler method). [b] As determined by blank run of initial volume of 5.00 ml. of supporting electrolyte. [c] Diffusion current of oxygen (column 3) corrected to original volume (column 5) plus diffusion current of B_{12a} (0.51 amp.). [d] Observed diffusion current of B_{12a} plus oxygen (column 4) corrected to original volume.

of operations, oxygen addition, stirring and recording of polarogram, was repeated continually until sufficient oxygen had been added to have combined with the vitamin present. Throughout the titration a faint stream of nitrogen was swept over the surface of the solution. The temperature was maintained at 25.0 ± 0.2°.

At the end of the titration the solution was transferred to a volumetric flask. The cell was rinsed with small portions of the standard oxygen solution, measured from the Machlett buret, until the volumetric flask was filled to the mark. The difference in the volume of the volumetric flask and the volume added during and after the titration gave the initial volume of B_{12r} before the titration. An aliquot of 0.200 ml. was taken for cobalt analysis.

Blank polarograms were recorded in a separate series of runs for the supporting electrolyte alone and were treated with successive portions of the standard oxygen solution. Typical polarograms are shown in Fig. 4.

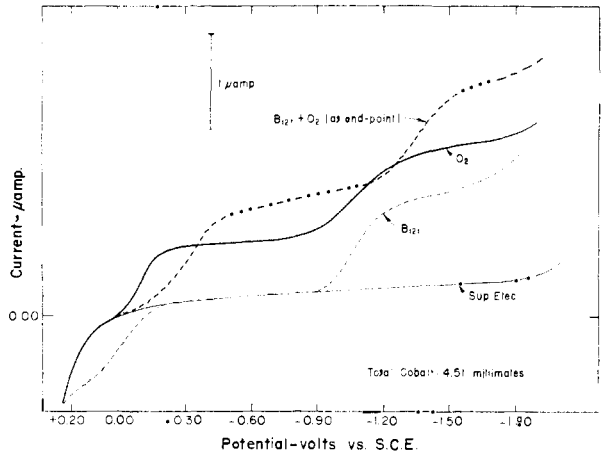

Fig. 4.—Polarograms of B_{12r}, oxygen, B_{12r} plus oxygen, and supporting electrolyte.

The diffusion current was measured on the polarograms recorded by the standard procedure. The diffusion currents obtained experimentally were corrected for the dilution as described previously. The results for a representative titration summarized in Table III and shown in Fig. 5 indicated presence of two end-points, at 1.17 ml. and at 3.48 ml. of standard oxygen solution.

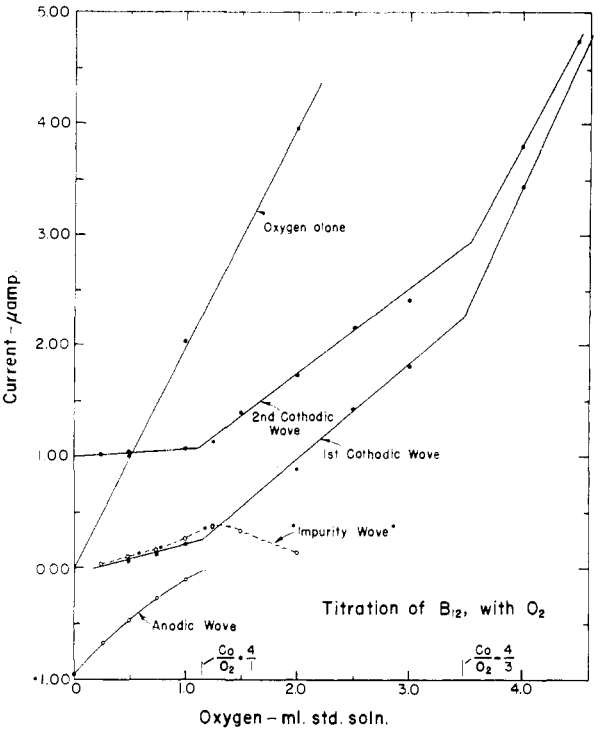

Fig. 5.—Amperometric titration of B_{12r} with standard oxygen solution.

Amperometric Titration of B_{12}.—Crystalline B_{12} was dissolved in 5.00 ml. of 0.1 *N* potassium sulfate in the polarograph cell. The solution was mixed and deaerated. The titration was carried out in the same manner as the titration of B_{12a} described earlier. Blank polarograms were obtained on the supporting electrolyte treated with the successive portions of the standard oxygen solution. The results of a representative titration are given in Table IV.

Results and Discussion

The treatment of B_{12a} with oxygen shifted the half-wave potentials of the first and second waves in the negative direction. In addition, the diffusion currents observed were less than the sum of the diffusion currents of B_{12a} and oxygen. The diffusion currents continued to be less to the point where one molecule of oxygen had been added for every two molecules of B_{12a}. Beyond this point, the diffusion current increased at the rate found for the addition of oxygen to the supporting electrolyte; see the titration curve of Fig. 3. The results of four titrations are shown in Table V. The polarogram obtained at the end-point is shown in Fig. 2. The first wave of the oxygen-bearing dimer is well defined with a half-wave potential of −0.22 v. (contrasted with −0.04 v. for the B_{12a} monomer) and the second wave is spread out with a half-wave potential of −1.24 v. (as contrasted to −1.02 v. for the monomer).

The reaction between B_{12r} and oxygen apparently takes place stepwise, and consists of a first step in which the cobalt is oxidized to the trivalent state

TABLE III

POLAROGRAPHIC BEHAVIOR OF SOLUTIONS OF OXYGEN AND B_{12r} PLUS OXYGEN

Initial volume of the supporting electrolyte, 4.97 ml.; initial concentration of B_{12r}, 0.906 mmolar.

Oxygen added: Vol. of stand. soln.,[a] ml.	Oxygen added: Quant., mmoles	Oxygen alone,[b] μamp.	Diffusion current obsd., μamp. B_{12r} plus oxygen: Anodic wave	1st cath. wave	Impur. wave	2nd cath. wave	Diffusion current obsd., μamp. Oxygen alone	Diffusion current calcd. to initial vol., μamp. B_{12r} plus oxygen: Anodic wave	1st cath. wave	Impur. wave	2nd cath. wave
0.00	0.000	0.00	0.95	0.00	0.00	1.00	0.00	0.95	0.00	0.00	1.00
.25	.232	.49	.53	.02	.04	0.99	0.51	.56	.02	.04	1.04
.50	.465	.94	.32	.07	.10	.96	1.03	.35	.08	.11	1.05
.75	.698	1.34	.23	.14	.16	.93	1.54	.26	.16	.18	1.07
1.00	.931	1.74	.07	.22	.22	.90	2.08	.08	.26	.26	1.08
1.25	1.165		.00	.32	.32	.91		.00	.40	.40	1.14
1.50	1.397	2.38	.00	.43	.26	1.02	3.10	.00	.56	.34	1.33
2.00	1.862	2.94	.00	.63	.10	1.26	4.12	.00	.88	.14	1.76
2.50	2.333		.00	.97	.00	1.44		.00	1.45	.00	2.16
3.00	2.793	3.94	.00	1.14	.00	1.59	6.30	.00	1.82	.00	2.55
4.00	3.724		.00	1.92	.00	2.11		.00	3.45	.00	3.80
4.50	4.190		.00	2.40	.00	2.56		.00	4.55	.00	4.86

[a] Concentration of the standard solution: (1) 30.2 p.p.m. (from the polarographic data and calculations using Ilkovic equation); (2) 29.8 p.p.m. (Winkler method). [b] As determined by blank run adding standard oxygen solution to 5.00 ml. of supporting electrolyte.

TABLE IV

POLAROGRAPHIC BEHAVIOR OF OXYGEN AND OF B_{12} PLUS OXYGEN

Initial volume of the supporting electrolyte, 5.00 ml.; initial concentration of B_{12}, 0.750 mmolar.

Oxygen added: Vol. of stand. soln.,[a] ml.	Oxygen added: Quant., mmoles	Dif. current, obsd., μamp.: Oxygen alone[b]	Dif. current, obsd., μamp.: B_{12} plus oxygen	Dif. current calcd. to initial vol., μamp.: Oxygen alone	Dif. current calcd. to initial vol., μamp.: B_{12} plus oxygen
0.00	0.000	0.00	0.00	0.00	0.00
0.50	.436	0.88	0.88	0.97	0.97
1.00	.872	1.74	1.71	2.09	2.05
1.50	1.308	2.42	2.42	3.15	3.15
2.00	1.744	2.88	2.87	4.03	4.01
2.50	2.180	3.34	3.32	5.01	4.98
3.00	2.620	3.77	3.74	6.02	5.97
3.50	3.050	4.12	4.05	7.00	6.89
4.00	3.490	4.51	4.45	8.12	8.00

[a] Concentration of the standard solution 27.9 p.p.m. Winkler method). [b] As determined by blank run.

TABLE V

TITRATION RESULTS OF B_{12a} WITH OXYGEN

Titration no.	Cobalt taken, mmoles	Oxygen required to reach end-point, mmoles	Ratio CO:O_2	Diffusion current,[a] amp.	"n" Electrons[b]
1	3.73	1.93	1.95	2.17	3.38
2	2.85	1.44	1.98	1.91	3.84
3	4.67	2.23	2.09	3.05	3.78
4	4.39	2.38	1.85	2.52	3.32

[a] Diffusion current at the end-point; corrected for dilution during titration. [b] Calculated by the Ilkovic equation, $n = i_d/(605\ CD^{1/2}m^{2/3}t^{1/6})$, in which i_d is the diffusion current at the end-point, C the millimolar concentration of B_{12a} dimer, D the diffusion coefficient of B_{12a} dimer (2.33×10^{-6} cm.^{2}/sec.), and $m^{2/3}t^{1/6}$ the capillary constant at −0.1 v. toward S.C.E. (1.875 mg.$^{2/3}$/sec.$^{1/6}$).

(conversion of B_{12r} to B_{12a}), and a second step in which the dimerization is effected. B_{12r} is characterized by an anodic wave of half-wave potential −0.04 v. toward the s.c.e. and a cathodic wave of half-wave potential −0.94 v. toward the s.c.e. In the early part of the titration with oxygen the anodic wave disappeared and was replaced by a cathodic wave characteristic of B_{12a}; the cathodic wave of B_{12r} ($E_{1/2}$ = −0.94 v.) did not change in height or position (this wave is essentially in the same position as the second cathodic wave of B_{12a} and presumably represents the same reaction, that is, the reduction of bivalent cobalt to univalent cobalt). This first reaction ended abruptly at the ratio B_{12r}:O_2 = 4:1. The color of the solution at this point had turned from brown to red-orange. Up to this point, the reaction apparently consisted simply of the oxidation of the cobalt from the bivalent to the trivalent state. Beyond the first end-point the two cathodic waves were shifted to more negative potentials and the behavior was identical with that observed in the titration of B_{12a} with oxygen. The second end-point occurred at the ratio B_{12a}:O_2 = 4:3, and thus the second part of the titration consisted in the dimerization produced by the union of one molecule of oxygen to two molecules of B_{12a}. The results of three titrations of B_{12r} with oxygen solution are shown in Table VI.

There is present in the polarogram of B_{12a} a small, unexplained wave of half-wave potential −0.55 v., the diffusion current for which represents about 0.25 electrons per molecule of B_{12a}. This wave is designated here for convenience as the impurity wavelet. On treatment of B_{12a} with oxygen, the impurity wavelet is shifted progressively to more positive potentials and decreased in height; finally, at the end-point of the titration, it disappears at the potential at which it would merge with the wave of the oxygen-bearing dimer (Co:O_2 = 2:1). The impurity wavelet is not present in the polarogram of B_{12r}. It appeared on the first addition of oxygen to B_{12r} and increased in height with each successive addition of oxygen, reaching a maximum at the first end-point (Co:O_2 = 4:1). Beyond the first end-point the impurity wavelet decreased in height, shifted toward the left, and

TABLE VI

TITRATION RESULTS OF B_{12r} WITH OXYGEN

Titration no.	Cobalt taken, mmoles	Oxygen required to reach end-point, mmoles 1st	2nd	Ratio Co:O₂ at end-points 1st	2nd	Diffusion current[a] at end-points, amp. 1st	2nd	"n" Electrons at end-point[b] 1st	2nd
1	4.51	1.11	3.26	4.08	1.38	1.10	2.92	0.63	3.74
2	5.25	1.32	..	4.03	..	1.31	..	.64	..
3	4.23	1.07	3.25	3.95	1.30	1.11	2.80	.65	3.82

[a] Diffusion current at the end-point; corrected for dilution. Data for run 2 were taken beyond the end-point. [b] Calculated by the Ilkovic equation; $n = i_d/(605\ CD^{1/2}m^{2/3}t^{1/6})$ in which i_d is the diffusion current at the end-point; C is the millimolar concentration of B_{12r}: (1) in calculations at the first end-point concentration of B_{12r} was used, (2) in calculations at the second end-point concentration that was equivalent to the B_{12a} dimer was used; D is the diffusion coefficient: (1) in the calculations at the first end-point the diffusion coefficient that of B_{12} (2.95×10^{-6} cm.²/sec.), (2) in the calculations at the second end-point the diffusion coefficient that of B_{12a} dimer (2.33×10^{-6} cm.²/sec.) was used; $m^{2/3}t^{1/6}$ is the capillary constant at -0.1 v. toward S.C.E. (18875 mg.$^{2/3}$ sec.$^{1/6}$).

merged with the wave of the dimer at the second end-point. That is, the behavior is identical to that of B_{12a} during this part of the titration.

The titration of B_{12} with standard oxygen solution showed no interaction between the two substances. The diffusion currents throughout the titration were simply those predicted by the addition of the diffusion currents of the two materials measured separately.

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The Molecular Weights and Dimensions of Some High-density Human Serum Lipoproteins

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Two high-density lipoprotein fractions were isolated by preparative ultracentrifugation. Sedimentation coefficient, apparent partial specific volume and molecular weight were measured centrifugally. The molecular weights at *p*H 6.7 were 1.75×10^5 for the lipoprotein with partial specific volume 0.867 and sedimentation coefficient 4.65 (HDL-3), and 4.0×10^5 for the lipoprotein with partial specific volume 0.905 and sedimentation coefficient 5.45 (HDL-2). Measurements at various solution densities and concentrations indicated that HDL-3 was a fairly homogeneous fraction.

Introduction

In 1949 Gofman, Lindgren and Elliot[4] showed that the boundary anomaly observed by McFarlane[5] and Pedersen[6] in ultracentrifugation of human serum could be interpreted as a pile up of lipoproteins at the albumin boundary. Subsequent work by Gofman and his associates[7] described the isolation of low density lipoproteins[8] by centrifugal techniques. The isolation and characterization of the high density lipoproteins has been reviewed by deLalla and Gofman.[9]

In a recent paper, Lindgren, Freeman, Nichols and Gofman[10] described a model for lipoprotein structures which featured a core of triglyceride, chloresterol and cholesteryl esters surrounded by an outer shell of high-density lipoproteins. It seemed of interest to characterize some of the high-density lipoproteins more precisely than has been done previously. Klainer and Kegeles[11] have described a modification of the Archibald[12] method for determining molecular weights by approach to sedimentation equilibrium. This modification permits determination of molecular weight from centrifugal data obtained over a period of a few hours.

The molecular weight data may be combined with sedimentation coefficients to calculate frictional factors and axial ratios of the macromolecules.[13] The partial specific volume, which is needed for the calculations, could be determined centrifugally by the method of Katz and Schachman.[14]

Experimental

Materials.—Following the nomenclature of deLalla and Gofman,[9] HDL-1, HDL-2 and HDL-3 are high-density

(1) This paper is taken in part from a dissertation submitted to the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy, January, 1957. Presented at the American Chemical Society Meeting, September, 1957, New York City.

(2) National Science Foundation Predoctoral Fellow, 1954–1955. U. S. Public Health Service, National Heart Institute, Predoctoral Fellow, 1955–1956.

(3) Arthur D. Little, Inc., Cambridge 42, Mass.

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(6) K. O. Pedersen, *J. Phys. Colloid Chem.*, **51**, 156 (1947).

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(8) Low-density lipoproteins are those macromolecules which float to the top of a centrifuge tube in the Spinco Model L Preparative Ultracentrifuge at a solution density of d^{26}_4 1.063 when centrifuged for 13 hr. at 40,000 r.p.m. in the 40.3 rotor. By this definition high-density lipoproteins are lipoproteins which sediment under the foregoing conditions.

(9) O. deLalla and J. W. Gofman, in Glick "Methods of Biochemical Analysis," Vol. I, Interscience Publishers, New York, N. Y., 1954, p. 459.

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(14) S. Katz and H. K. Schachman, *Biochim. Biophys. Acta*, **18**, 28 (1955).