

cation, the value of the dissociation constant is approximately that which might have been expected for an ion-pair in which the distance of closest approach is determined by the field around the three smaller alkyl groups.

2. **Nitrobenzene.**—In Table IV are given the limiting conductances of three quaternary ammonium nitrates containing long hydrocarbon chains in the cation. The cation conductances are given in column 3 and have been calculated on the assumption that the nitrate ion has a conductance of 22.6.⁶

TABLE IV
LIMITING CONDUCTANCES OF ELECTROLYTES IN NITROBENZENE

Salt	Λ_0	Λ_0^+
$(n\text{-C}_{18}\text{H}_{37})_2(\text{CH}_3)_2\text{NNO}_3$	30.7	8.1
$(n\text{-C}_{18}\text{H}_{37})(n\text{-C}_4\text{H}_9)_3\text{NNO}_3$	31.5	8.9
$(n\text{-C}_{18}\text{H}_{37})(\text{C}_6\text{H}_5\text{N})\text{NO}_3$	32.9	10.3

The above Λ_0 values for the three salts have been obtained by extrapolation of the plots shown in Fig. 2, in which values of Λ_0 are plotted against values of $C^{1/2}$. These plots are linear over a con-

siderable range of concentrations although the slopes of the lines are somewhat greater than the theoretical; this is doubtless due to ion-pair formation. Ion conductances decrease with increasing number of carbon atoms in the cation but rather less than one might otherwise expect.

V. Summary

1. The conductance of octadecyltrimethylammonium and octadecyltributylammonium nitrates in ethylene chloride and of octadecyltributylammonium, dioctadecyldimethylammonium and octadecylpyridonium nitrates in nitrobenzene have been measured.

2. These long chains salts are normal electrolytes over the concentration range studied in both ethylene chloride and nitrobenzene.

3. Limiting conductances and dissociation constants have been evaluated in ethylene chloride and limiting conductances in nitrobenzene.

4. Ion conductances have been evaluated in both solvents.

PROVIDENCE, R. I.

RECEIVED DECEMBER 16, 1947

[CONTRIBUTION FROM THE METCALF RESEARCH LABORATORY OF BROWN UNIVERSITY]

Properties of Electrolytic Solutions. XXXIII. The Conductance of Some Salts in Acetone at 25°¹

BY MYRON B. REYNOLDS² AND CHARLES A. KRAUS

I. Introduction

Acetone is a solvent that differs markedly in type from those previously investigated in this Laboratory; it has a dielectric constant of 20.5 and is a fair solvent for a number of uni-univalent inorganic salts. Solutions in this solvent have been investigated earlier by several different investigators³ but with the exception of Lannung, who was chiefly concerned with solubilities, their results are uncertain because of the high conductance of the solvent which introduced large and often uncertain corrections with solutions of low concentration. In this connection, it may be pointed out that, since the only laws that are known to apply to electrolytic solutions are of limiting type, it is of particular importance that experimental errors be kept at a minimum at low concentrations. *If a solvent cannot be adequately purified, there is little point in carrying out conductance measurements.*

(1) This paper comprises a portion of a thesis presented by Myron B. Reynolds in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1947.

(2) Anthony Fellow at Brown University, 1946-1947; present address: Research Laboratory, General Electric Company, Schenectady, N. Y.

(3) (a) Walden, Ulich and Busch, *Z. physik. Chem.*, **123**, 429 (1926); (b) Lannung, *ibid.*, **161A**, 255 (1932); (c) Hughes and Hartley, *Phil. Mag.*, **15**, 610 (1933); and others.

In the present investigation, the solvent has been purified to a point where correction for solvent conductance was negligible. The electrolytes investigated were, for the most part, salts of the tetrabutylammonium ion with various negative ions. The picrates of lithium, sodium and potassium were measured as were also potassium iodide and thiocyanate. It may be noted that numerous salts which have been measured by earlier investigators were found to be too difficultly soluble in the pure solvent to permit of ready measurement.^{3b}

Owing to uncertainties in the value of the physical constants of acetone, these (dielectric constant, viscosity and density) were redetermined.

The results of conductance measurements have been treated by the method of Fuoss⁴ and values of the limiting conductance, Λ_0 , and the dissociation constant, K , have been derived. Ion conductances have been evaluated according to the method of Fowler.⁵

II. Experimental

Apparatus.—Conductance measurements were carried out as described in earlier papers of this series, using a Jones type a. c. bridge and Erlenmeyer conductance cells with bright platinum electrodes. All measurements were carried out at 25 ± 0.01°.

The dielectric constant of acetone was measured at 25°,

(4) Fuoss, *THIS JOURNAL*, **57**, 488 (1935).

(5) Fowler and Kraus, *ibid.*, **62**, 2237 (1940).

using a parallel substitution type a. c. bridge⁶ and a cell especially designed to minimize lead errors. Solvent conductance was also measured with this bridge.

Solvent viscosity was measured at 25°, using a modified Ostwald viscometer calibrated against water and benzene. Solvent density was determined by means of an Ostwald-Sprengel pycnometer.

Materials.—Commercial acetone⁷ was dried by agitation over calcium chloride and then distilled twice from activated alumina pellets.⁸

In each distillation, the middle fraction only was saved and the final distillation was preceded by a few hours of refluxing. The conductance of acetone prepared in this way was in the range of 1 to 2 × 10⁻⁹ reciprocal ohm centimeters; corrections for solvent conductance were thus negligible.

Tetra-*n*-butylammonium triphenylborofluoride was prepared by Dr. G. L. Brown, of this Laboratory, and purified by successive recrystallizations from ether-ethanol mixtures. It was dried at room temperature *in vacuo*; m. p. 165–166°.

Tetra-*n*-butylammonium picrate from laboratory stock was recrystallized from ethanol, and dried *in vacuo* at slightly elevated temperature; m. p. 89°.

Tetra-*n*-butylammonium iodide from laboratory stock was purified by recrystallization from nitromethane and dried *in vacuo* at room temperature; m. p. 146°.

Tetra-*n*-butylammonium bromide was prepared by treating *n*-butyl bromide with tri-*n*-butylamine in ethanol solution at about 70°. The crude salt was recrystallized several times from ethyl acetate and twice from benzene-petroleum ether mixtures; a portion was recrystallized from an ether-ethyl acetate mixture. Both samples were dried to constant weight *in vacuo* at 50 to 60°; m. p. 118°.

Tetra-*n*-butylammonium nitrate was prepared by Dr. H. L. Pickering, of this Laboratory, purified by recrystallizations from benzene, and dried *in vacuo* at room temperature; m. p. 119°.

Tetra-*n*-butylammonium perchlorate was prepared in this Laboratory by Dr. L. E. Strong. Further purification was effected by recrystallization from ether-acetone mixtures, followed by drying *in vacuo* at room temperature; m. p. 213°.

Tetraethylammonium picrate, furnished by Dr. C. J. Carignan, of this Laboratory, was recrystallized from ethanol and dried to constant weight *in vacuo* at 65–80°.

Tetramethylammonium triphenylborofluoride from laboratory stock was recrystallized from acetone and from acetone-ethanol mixtures and dried *in vacuo* at room temperature; m. p. 186°.

Tetramethylammonium fluoride was prepared in this Laboratory by Dr. C. J. Carignan. The salt was purified by precipitation from ethanol solution by addition of ethyl acetate and also by recrystallization from ethanol-acetone mixtures. Samples were dried to constant weight at 50° *in vacuo*; m. p. 268–269°, with decomposition.

Lithium picrate, prepared in this Laboratory, by Dr. C. J. Carignan, was purified both by recrystallizations from ethanol-nitromethane mixtures and from acetone-nitromethane mixtures. The salt was dried *in vacuo* at 80°.

Sodium picrate was prepared by neutralizing an ethanol solution of picric acid with aqueous sodium hydroxide solution. The resulting salt was recrystallized several times from ethanol-water mixtures and dried to constant weight *in vacuo* at 70–80°.

Pure samples of potassium picrate were available from laboratory stock and were dried to constant weight *in vacuo* at 65°.

Reagent grade potassium iodide was recrystallized from water and also from water-ethanol mixtures. The recrystallized samples were dried *in vacuo* at 70°.

Reagent grade potassium thiocyanate was further purified

by recrystallization from water and from acetone-water mixtures, and dried *in vacuo* at 50°.

III. Results

Physical Constants.—The dielectric constant of acetone was determined with thirteen different

TABLE I

CONDUCTANCES OF VARIOUS SALTS IN ACETONE AT 25°

Tetra- <i>n</i> -butylammonium triphenylborofluoride		Tetra- <i>n</i> -butylammonium picrate	
C × 10 ⁴	Δ	C × 10 ⁴	Δ
0.4517	130.0	0.4237	147.9
0.8237	128.5	0.9049	146.0
1.584	126.0	1.717	143.3
3.213	122.4	3.588	139.2
7.003	116.8	7.129	133.7
15.47	109.1	17.02	124.4
Tetra- <i>n</i> -butylammonium perchlorate		Tetra- <i>n</i> -butylammonium nitrate	
0.3115	178.4	0.4074	181.6
0.7118	176.2	0.8190	178.8
1.479	172.5	1.291	176.2
3.142	167.3	2.879	169.6
6.860	159.3	8.414	155.4
20.16	144.0	25.27	134.3
Tetra- <i>n</i> -butylammonium bromide		Tetra- <i>n</i> -butylammonium iodide	
0.3287	177.8	0.3086	175.4
0.6875	174.8	0.4963	174.1
1.523	169.1	1.019	171.2
3.504	159.7	2.674	164.5
9.188	143.6	9.215	149.1
21.48	125.1	25.98	131.1
Tetraethylammonium picrate		Tetramethylammonium triphenylborofluoride	
0.4129	171.9	0.4044	160.2
0.7731	170.1	0.8572	157.6
1.535	167.2	1.606	154.2
3.188	162.8	3.371	148.6
7.044	156.0	7.145	140.3
19.19	144.7	18.30	126.3
Tetramethylammonium fluoride		Lithium picrate	
0.3422	172.2	0.3453	150.2
0.6672	166.1	0.7875	144.0
1.385	155.5	1.397	137.5
2.880	140.0	3.003	125.2
6.492	118.4	6.398	109.3
		20.92	79.79
Sodium picrate		Potassium picrate	
0.5370	154.0	0.2834	161.7
1.073	148.2	0.7824	157.6
2.348	138.0	1.981	150.9
4.878	124.9	3.183	145.9
10.57	107.9	9.448	129.7
21.99	90.35	23.14	111.8
Potassium iodide		Potassium thiocyanate	
0.4962	186.8	0.2722	196.8
1.086	183.6	0.8890	191.5
3.142	175.8	2.748	181.3
6.124	168.4	7.564	165.5
13.11	156.7	18.45	146.3
24.41	145.4		

(6) L. E. Strong, Thesis, Brown University, 1940.

(7) The acetone was kindly donated by the Tennessee Eastman Corporation.

(8) Harshaw AL-4 "Catalyst Pellets."

samples the specific conductance of which varied between 0.51 and 1.7×10^{-9} . The average value found for the dielectric constant was 20.47, with a mean deviation of 0.05 and a maximum deviation of 0.2.

The viscosity was determined with sixteen different samples of acetone of which only two had a specific conductance above 2×10^{-9} ; thirteen of these samples were identical with those used in the dielectric constant measurements. The average value of the viscosity in poise was found to be 3.040×10^{-3} with a mean deviation of 0.008×10^{-3} .

The density of acetone was determined to be 0.7845. All the above values were at 25°.

Conductances.—Equivalent conductances, Λ , and concentrations, C , expressed in moles of salt per liter of solution, are given in Table I. Two or more series of measurements were carried out with salt samples resulting from successive recrystallizations and the salts were assumed to be pure when conductances for successive recrystallizations for a given salt agreed within the limit of experimental error, 0.1%.

IV. Discussion

The data of Table I were analyzed by the method of Fuoss by plotting values of F/Λ against values of $c\Delta f^2/F$. The plots are shown in Figs. 1, 2 and 3, on which appear values for two series of measurements for each salt. Inspection of the plots will show that Λ_0 may be determined with considerable precision, particularly in the case of the stronger salts. All plots are linear within the limit of experimental error up to about $7 \times 10^{-4}N$.^{8a} The slopes of the plots serve in the evaluation of the dissociation constant, K , of the ion pairs; the curves are the steeper, the lower the constant. Values of Λ_0 and K as determined

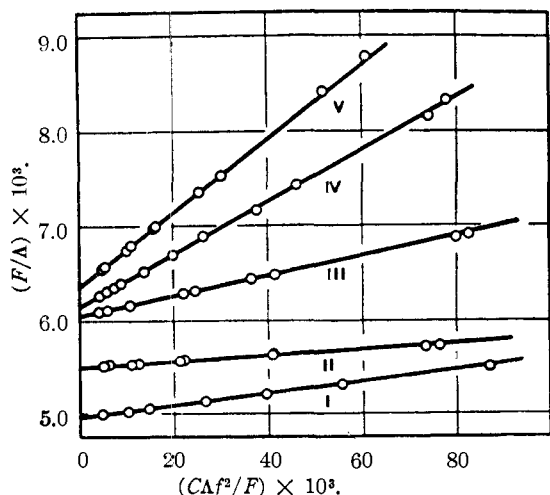


Fig. 1.—Fuoss plots for salts in acetone: I, KCNS; II, *n*-Bu₄NCIO₄; III, KP_i; IV, NaP_i; V, LiP_i.

(8a) The critical concentration for acetone is 2.8×10^{-3} [Fuoss, THIS JOURNAL, 57, 2804 (1935)].

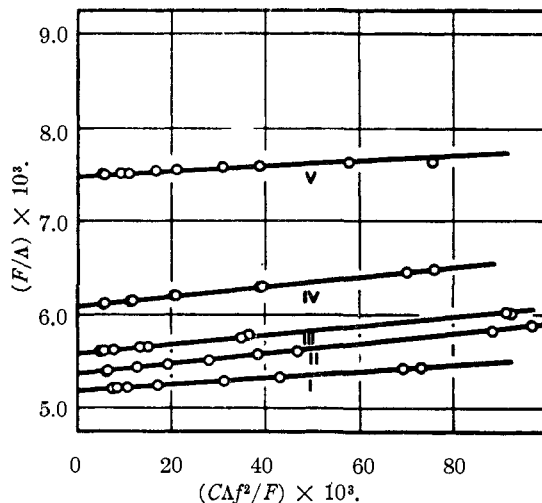


Fig. 2.—Fuoss plots for salts in acetone: I, KI; II, *n*-Bu₄NNO₃; III, *n*-Bu₄NI; IV, Me₄NFBPh₃; V, *n*-Bu₄NFBPh₃.

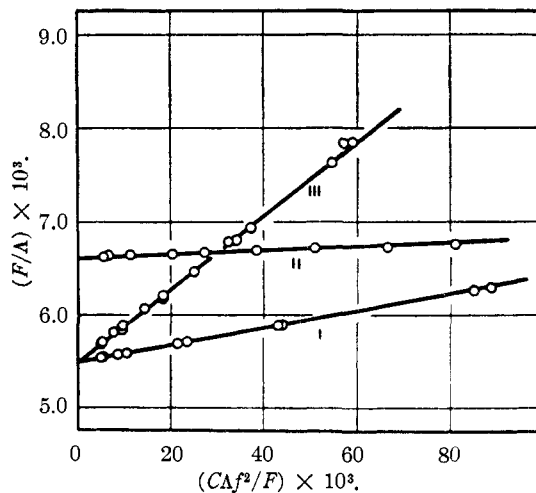


Fig. 3.—Fuoss plots for salts in acetone: I, *n*-Bu₄NBr; II, *n*-Bu₄NP_i; III, Me₄NF.

by means of the plots are given in columns 2 and 3 of Table II.

TABLE II
CONSTANTS OF VARIOUS SALTS IN ACETONE AT 25°

Salt	Λ_0	$K \times 10^3$	Λ_0^+	Λ_0^-
(<i>n</i> -C ₄ H ₉) ₄ NFB(C ₆ H ₅) ₃	134.2	19.7	67.1	67.1
(<i>n</i> -C ₄ H ₉) ₄ NP _i	152.4	22.3	..	85.3
(<i>n</i> -C ₄ H ₉) ₄ NCIO ₄	182.4	9.58	..	115.3
(<i>n</i> -C ₄ H ₉) ₄ NNO ₃	187.2	5.46	..	120.1
(<i>n</i> -C ₄ H ₉) ₄ NBr	183.0	3.29	..	115.9
(<i>n</i> -C ₄ H ₉) ₄ NI	179.4	6.48	..	112.3
(C ₂ H ₅) ₄ NP _i	176.5	17.5	91.2	..
(CH ₃) ₄ NFB(C ₆ H ₅) ₃	165.1	6.93	98.0	..
(CH ₃) ₄ NF	183	0.77	..	85
LiP _i	158.1	1.03	72.8	..
NaP _i	163.7	1.35	78.4	..
KP _i	165.9	3.43	80.6	..
KI	192.8	8.02	80.5	..
KCNS	201.6	3.83	..	121.0

Ion Conductances.—Ion conductances have been evaluated by the method of Fowler according to which the two ions of tetrabutylammonium triphenylborofluoride are assumed to have the same conductance. The value so found for the tetrabutylammonium ion is 67.1 and the ion conductances recorded in columns 4 and 5 of Table II are based on this value.

The conductance of negative ions is markedly higher than that of comparable positive ions. Thus the conductance of the perchlorate ion is 115.3 while that of the tetramethylammonium ion is only 98.0. The conductance of the alkali metal ions decreases markedly with decreasing atomic weight, the change from sodium to lithium being particularly marked. Considering the tetraethylammonium ion with nine atoms other than hydrogen and a conductance of 91.2, the low conductance of the lithium ion (72.8) indicates a high degree of interaction with the solvent dipoles. There may be actual solvation of the lithium ion, but we have no satisfactory means of distinguishing between solvation and simple interaction of the charge on the ion with the dipoles of the solvent molecules. The low conductance of the tetramethylammonium ion in comparison with that of the perchlorate ion cannot well be ascribed to solvation in the strict sense of the term.

The high conductance of the thiocyanate ion, as also that of the nitrate ion, is worthy of note. The bromide ion has a slightly higher conductance than the iodide ion, but the fluoride ion has a very low conductance. But, even here, we find the conductance of an exceptionally small negative ion markedly greater than that of the ion of the much larger potassium atom, 85 as against 80.6.

There is only one case among the salts in the table where ion conductances as derived from different pairs of ions may be compared. The conductance of the potassium ion as derived from its picrate is 80.6 while that derived from the iodide is 80.5. The difference lies within the limit of experimental error.

There is only scanty material in the literature that can be employed for purposes of comparison. Seemingly, the most reliable conductance measurements with solutions of salts in acetone are due to Ross Kane but, unfortunately, the details of his measurements are unavailable, and only rounded ion conductances at 25° have been reported.⁹

In the case of several salts (KI and Et₄N⁺Pi⁻), our conductance values are in good agreement with those of Ross Kane. In other instances, the difference usually lies between 0.5 and 1.0%. If Ross Kane's limiting conductance values were obtained by extrapolation of the usual square root plot, discrepancies might well be accounted for by extrapolation errors.

Dissociation Constants.—In accord with the dielectric constant of acetone (D 20.47), solu-

tions of salts in this solvent are fairly highly ionized, but all show marked ion pair association so that the evaluation of Λ_0 by extrapolation of the $\Lambda-\sqrt{C}$ plot is not permissible.

Since the energy necessary to separate a pair of ions is a function of the distance between centers of charge in the ion pairs, it follows that salts having large ions have relatively large dissociation constants while salts with small ions will have small constants. Since large ions have lower conductances than small ions, we should expect that salts having ions of lower conductance would have larger dissociation constants.

However, such a simple relation does not hold, chiefly, because of certain specific factors:

1. The effective size of an ion may be due either to a large structure, as in the case of the tetrabutylammonium ion, or, on the other hand, it may be the result of interaction of a small ion with the molecules (dipoles) of the solvent. In the latter case, the solvent molecules may, in some instances, be definitely attached to the ion; in others, the structure may be a very loose one; and, in still others, both types of interaction may be involved. The smallest ions, lithium and fluoride, have very low conductances but yield relatively small dissociation constants. Evidently, solvent molecules, which may be attached to the free ions, are largely lost in the ion pairs. The nitrate ion has a much higher conductance than the bromide ion, yet its tetrabutylammonium salt is much stronger than the corresponding bromide.

2. Steric effects have a marked influence on the dissociation constant. Thus, potassium iodide is much stronger than potassium picrate, yet the constant of tetrabutylammonium picrate is three times that of the iodide. Then again, the constant for tetrabutylammonium picrate is but little greater than that of the corresponding tetraethylammonium salt. This indicates that the picrate ion penetrates into the shell of butyl groups about the nitrogen atom to a depth that approximates the dimensions of the tetraethylammonium group.

As was shown in an earlier paper of this series,¹⁰ a quantity " a ," which may be interpreted as an approximation to the distance between charges in the ion pairs, may be computed from the dissociation constant. For the salts whose constants are given in Table II, the value of " a " varies from 2.42 Å. for tetramethylammonium fluoride to 9.48 Å. for tetrabutylammonium picrate. That interaction with solvent molecules is a factor in determining ion-pair dimensions in acetone, even in the case of lithium picrate, is shown clearly by the results with the same salt in nitrobenzene (D 34.5).¹¹ The " a " distance for this salt in the two solvents is, respectively, 2.55 and 0.62 Å. The dissociation constant of 1.03×10^{-3} for lithium picrate in acetone of dielectric constant 20.5 as against one of 6×10^{-8} in a solvent of dielectric

(9) Murray-Rust, Gatty, MacFarlane and Hartley. *Ann. Repts. Chem. Soc.*, **27**, 351 (1930).

(10) Fuoss and Kraus, *This Journal*, **55**, 1019 (1933).

(11) Witschonke and Kraus, *ibid.*, **69**, 2472 (1947).

constant 34.5 can only be due to interaction between the ions (in the ion pairs) with the acetone molecules, on the one hand, and lack of such interaction with nitrobenzene molecules, on the other. A comparison of sodium and potassium picrates in acetone and nitrobenzene shows that these salts behave in a manner similar to that of lithium picrate. The Walden conductance viscosity product for the sodium and potassium ions in acetone is 0.238 and 0.242, respectively, while in nitrobenzene¹¹ it is 0.295 and 0.322. Thus, the free ions in this solvent are of very nearly the same size. The corresponding K values $\times 10^4$ are, respectively, 13.5 and 34.3 in acetone and 0.28 and 6.86 in nitrobenzene. The effective size of these ions in the ion pairs is much smaller in nitrobenzene than in acetone and is progressively more so as the (lattice) ion is smaller.

V. Summary

A simplified procedure for the purification of

acetone has been developed, making use of activated alumina pellets.

The density, viscosity and dielectric constant of purified acetone at 25° have been determined.

Conductance data have been obtained for fourteen different salts in acetone solution at 25°.

Limiting conductances and dissociation constants have been calculated for these salts, using the extrapolation method of Fuoss.

Limiting ion conductances have been computed by the method of Fowler.

Anion conductances have been found to be, in general, greater than corresponding cation conductances, suggesting specific solvent interaction with cations.

The conductance of the fluoride ion has been found to be markedly lower than that of the other halide ions and abnormally low for an anion.

PROVIDENCE, R. I.

RECEIVED JANUARY 14, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE DEPARTMENT OF AGRICULTURAL BACTERIOLOGY UNIVERSITY OF WISCONSIN]

The Effect of Various Gases on Nitrogen Fixation by *Azotobacter*¹

BY DOROTHY M. MOLNAR, R. H. BURRIS AND P. W. WILSON

Molecular hydrogen was demonstrated by Wilson and his associates^{2,3,4} to inhibit nitrogen fixation, specifically and competitively, in both free-living *Azotobacter* and the symbiotic system of red clover plus *Rhizobium*. It has been suggested⁵ that the inhibition might be primarily a physical effect dependent on the relative adsorption of the two gases on the surface of the nitrogen-fixing enzyme. The observation that the enzyme-substrate dissociation constants of hydrogen and nitrogen in *Azotobacter* have essentially the same ratio as their van der Waals constants offers some support for this view.⁶ Although this may be only fortuitous, an examination of the effect of gases with different physical properties, appears to be desirable.

Experimental

Methods.—Cultures of *Azotobacter vinelandii* were maintained by daily transfer to 50 ml. of Burk's medium³ in 500-ml. Erlenmeyer "shake" flasks. Weekly tests for purity were made by microscopic examination (Gram stain) and by inoculation of beef extract-peptone broth.⁶ For the microrespirometer studies, ten drops of a culture (seventeen to nineteen hours) was diluted with 40 ml. of sterile Burk's medium, and 2 ml. transferred to the res-

piration flask. The conventional techniques for supplying different gas mixtures in respiratory experiments were followed.⁷ Manometer readings were taken at half-hour intervals over a period of five hours, at 30°. The gas mixtures were: p_{N_2} , 0.2 atm.; p_{O_2} , 0.2 atm.; helium, argon, neon, hydrogen, ethane, or nitrous oxide, 0.6 atm. At a partial pressure of nitrogen of 0.2 atm. the rate of fixation is about 95% of maximum (in the absence of a competitive inhibitor, such as H₂), and small variations in the pressure (± 0.02 atm.) cause little change. The partial pressure of oxygen likewise is near optimum; to insure that it was kept reasonably constant, the oxygen used in respiration was replaced periodically. The mixtures were made from ordinary cylinder gases (about 98–99% pure) with the exception of the nitrous oxide, which was the grade used for anaesthesia. Errors arising from variation in the composition were reduced by preparing separate mixtures for the replicate experiments.

In a few trials, the conclusions from the microrespiration data were checked by estimating the initial and final total nitrogen in representative flasks by a modification of the micromethod of Johnson.⁸ One macro experiment was made: Ten ml. of an eighteen-hour culture was diluted with 300 ml. of sterile Burk's medium, and 25-ml. aliquots were pipetted into sterile 250 ml. Erlenmeyer flasks. Each flask contained a tube of potash to absorb respiratory carbon dioxide and was made gas-tight with a rubber stopper fitted with an inlet tube. After the desired gas mixture was supplied through the inlet tube, the flasks were incubated in a conventional shaking apparatus at 30° for fifteen hours, then total nitrogen determined on aliquots by the micromethod.

Results and Discussion

The van der Waals constant a for the gases tested varied from 0.00007 (helium) to 0.01074

(7) Umbreit, Burris and Stauffer, "Manometric Techniques and Related Methods for the Study of Tissue Metabolism," Burgess Publishing Co., Minneapolis, Minn., 1945.

(8) Johnson, *J. Biol. Chem.*, **137**, 575 (1941).

(1) Supported in part by grants from the Rockefeller Foundation and from the Research Committee of the Graduate School from funds provided by the Wisconsin Alumni Research Foundation.

(2) Wilson, "The Biochemistry of Symbiotic Nitrogen Fixation," University of Wisconsin Press, Madison, Wisconsin, 1940.

(3) Wyss and Wilson, *Proc. Natl. Acad. Sci. (U. S.)*, **27**, 162 (1941).

(4) Wyss, Lind, Wilson and Wilson, *Biochem. J.*, **35**, 845 (1941).

(5) Burk and Burris, *Ann. Rev. Biochem.*, **10**, 587 (1941).

(6) Wilson and Burris, *Bact. Rev.*, **11**, 41 (1947).