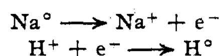
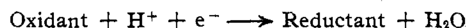
nitroguanidine in liquid ammonia, yields of 60 to 70% were obtained.

While the ammonium salts of strong acids have been used for decomposition of sodium-liquid ammonia reduction products,[2] this work appears to be the first example in which an acidic solution of liquid ammonia was used to change the environmental condition of the liquid ammonia solvent media during reduction. The importance of the environmental conditions of the solvent media for the reduction of nitroguanidine has been demonstrated.[9] The mechanism will become clear when it is remembered that the ammonium ion is an ammoniated hydrogen ion



$$Na^\circ \longrightarrow Na^+ + e^-$$
$$H^+ + e^- \longrightarrow H^\circ$$

if, however, an oxidant be present



$$\text{Oxidant} + H^+ + e^- \longrightarrow \text{Reductant} + H_2O$$

It, therefore, appears probable that liquid ammonia could also serve as a solvent medium for catalytic hydrogenations in which molecular hydrogen is introduced into a liquid ammonia solution of an oxidant in the presence of a catalytically active metal. We are at present engaged in determining the usefulness of liquid ammonia as a solvent medium for catalytic hydrogenations, and in extending the mechanism of the reduction and behavior of nitroguanidine in that solvent.

(9) Lieber and Smith, THIS JOURNAL, 58, 2170 (1936).

BROOKLYN, NEW YORK RECEIVED MARCH 26, 1937

Micro Analysis for Exchangeable Hydrogen

BY WILLIAM H. HAMILL

A technique has been developed for the determination of exchangeable hydrogen with 2–5 mg. of substance (water soluble, non-volatile) and 50–100 mg. of heavy water (98%) which is correct within 3%. The determination depends upon the decrease in density of the heavy water, due to exchange, as measured by a small quartz float[1,2] controlled by temperature at constant pressure. The density of the float was determined with potassium chloride solution, part of which was used for a pycnometric density determination at the same temperature as that required for flotation equilibrium.

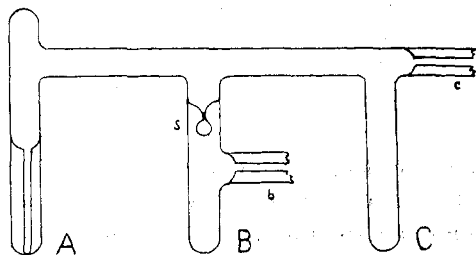


Fig. 1.—Exchange vessel.

Exchange analyses were made in a Pyrex vessel, hereafter referred to as the still (Fig. 1). The float was introduced into the capillary A, the substance into B and the heavy water, in a sealed ampoule, into C. The still was evacuated to about one micron through b and c and sealed. The heavy water was released by immersing C in dry ice, the freezing water shattering the ampoule, and distilled into A for a density measurement. The water was then distilled into the bulb-seal which was broken by freezing in dry ice.[3] The substance was dissolved and the water distilled off quickly at room temperature by cooling C with dry ice. The water was then distilled into A and the density redetermined. This process was repeated to ensure complete solution and exchange.

The number of exchangeable hydrogen atoms per molecule of substance was calculated from equation (1) with a parallel experiment upon a reference substance to minimize errors arising from exchange with glass, temperature coefficient of density of heavy water, etc. Three determinations with urea served for reference.

$$n_1/n_2 = W_1 M_1 \Delta t_1 S_2 N_2 / W_2 M_2 \Delta t_2 S_1 N_1 \quad (1)$$

n = number of exchangeable hydrogen atoms per molecule
S = weight of substance
M = molecular weight of substance
N = mole fraction D_2O after exchange
W = weight of heavy water
Δt = difference in flotation temperatures due to exchange

The results of these analyses appear in Table I and refer to immediate exchange (three minutes) at room temperature except as noted. Ogawa's[4] observation of slow exchange for urea (60% in five minutes) was not confirmed. Hydrogen in the methylene group of malonic acid has been shown

(1) The float, about 8 mm. × 0.6 mm., is maintained upright by means of a quartz bead at one end. The float accelerates 0.01 cm. sec./1° at 30–35°. The effect of pressure upon the density of the float, with respect to heavy water, is only $1(10)^{-5}/1$ atm.

(2) A Pyrex micro float has been employed by Goldfinger and Scheepers, *Compt. rend.*, 198, 1916 (1934).

(3) This seal is easy to make and positive in action, not failing once. The water freezes quickly at the constriction, sealing the bulb which is broken within two minutes.

(4) Ogawa, *Bull. Chem. Soc. Japan*, 11, 367 (1936).

to exchange[5] completely in five hours at 100°. In the present work the first two (carboxyl) hydrogen atoms were found to exchange at once, the other two more slowly with a half time of fifteen minutes. The sixth hydrogen atom in histidine hydrochloride apparently is due to a rapidly established equilibrium which corresponds

$$—NH—CH{=}N— \rightleftharpoons —N{=}CH—NH—$$

to known methyl derivatives. The fifth slowly exchanging hydrogen atom in vitamin B_1 hydrochloride is not accounted for but agrees with an observed[6] second titratable equivalent of acid in the vitamin with a slow approach to equilibrium. The rate of this slow exchange was measured with 3.2 mg. of vitamin and 76 mg. of heavy water at 37°. Initial, final and four intermediate observations gave a first order velocity constant (min.$^{-1}$ $\log_{10}$) $k = 4.3(10)^{-3}$.

This method is more tedious than the one recently described by Williams[7] but has the principal advantage of distinguishing between active hydrogen, as in hydroxyl and amino groups, and labile, slowly exchanging hydrogen, as in the methylene group of malonic acid. Also, since evacuation of the still precedes exchange there is no difficulty with hygroscopic substances. The sensitivity is high, 10^{-5} equivalent of substance in 50 mg. of heavy water lowering the flotation temperature by 0.3° at 30°.

TABLE I

Substance	n
Urea	(4.00)
Glycine	3.13
Histidine, HCl	6.07, 6.36
Vitamin B_1HCl natural	3.94[a,b] 4.83
Vitamin B_1HCl synthetic	3.6[a,c] 4.5
Hydroquinone	1.95
Sodium formate	0.00
Succinic acid	2.14, 2.06
Malonic acid	2.0[a] 3.99

[a] The first value is due to immediate exchange, the second is a final value following an additional slow exchange. [b] The author is indebted to Dr. L. R. Cerecedo and Dr. D. J. Hennessy for the natural vitamin which was extracted from rice polishings. [c] The difference in values between natural and synthetic vitamin may be due to mechanical loss during evacuation in this experiment.

FORDHAM UNIVERSITY
NEW YORK, N. Y.
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(5) Wynne-Jones, *Chem. Rev.*, **17**, 115 (1935).
(6) Williams and Ruehle, THIS JOURNAL, **57**, 1856 (1935).
(7) Williams, *ibid.*, **58**, 1819 (1936).

A Rapid Method for the Determination of Lactoflavin in Milk[1]

BY C. H. WHITNAH, BERNICE L. KUNERTH AND M. M. KRAMER

The determination of lactoflavin (vitamin G) by new fluorimetric methods[2] is complicated by elaborate preparation recommended for the sample.[3] It had been observed in this Laboratory that the trichloroacetic acid serum from milk used for vitamin C titrations[4] often had a greenish color whereas the mercuric nitrate serum used for the determination of sugar was colorless. This suggested that fluorimetric tests for flavin might be applied to the former serum.

The following procedure has been used. Add 15 ml. of 10% trichloroacetic acid to 10 ml. of milk, let stand thirty to sixty minutes, centrifuge five minutes at about 2000 r. c. f. Neutralize[5] 10 ml. of the resulting serum, with methyl orange as indicator, and dilute until the sample can be matched in the light of an Eveready Fluoray lamp,[2] with standard flavin solutions (Labco PX grade) containing 0.12 to 0.06 gamma of flavin per ml. Calculate flavin content on the basis of dilutions made. Dilutions until the portions read contain less than 0.12 gamma per ml. seem essential as the values for stronger solutions are easily underestimated.

It was repeatedly shown that a sample of milk tested the same on successive days. Differences between milk samples from different cows were also found to be consistent.

The method was checked by recovery experiments. Duplicate samples of milk were reinforced with lactoflavin (measured amounts of the standard solution) to contain approximately 2 and 3 times the original lactoflavin content. Values secured by calculation and by determination with the Fluoray lamp compared as follows.

	Lactoflavin per ml. Calcd. γ	Lactoflavin per ml. Determined by lamp γ	Diff. from calcd. value per ml. γ	Diff. from calcd. value per ml. %
(1) Milk	..	1.34	...	..
(2) 9 ml. milk + 11.44 γ flavin made up to 10 ml.	2.35	2.12	0.23	10
(3) 9 ml. milk + 22.87 γ flavin made up to 10 ml.	3.49	3.19	0.30	9

(1) Contribution No. 233, Department of Chemistry and No. 67, Department of Home Economics.
(2) Supplee, Ansbacher and Bender, *J. Biol. Chem.*, **110**, 365 (1935).
(3) Kuhn, György and Wagner-Jauregg, *Ber.*, **66**, 1034 (1933).
(4) Whitnah and Riddell, *J. Dairy Sci.*, **20**, 9 (1937).
(5) Kuhn and Moruzzi, *Ber.*, **65**, 888 (1932).