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.

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RECEIVED MARCH 29, 1937

(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	Determined by lamp γ	Diff. from calcd. value per ml. γ	%
(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).

Recoveries were only 10% less than the calculated values.

Other composite milk samples have been used for biological assay by the Bourquin-Sherman[6] method, shown[7] to measure the flavin factor. The rats, kept for the customary eight-week period, produced satisfactory composite growth curves. Estimates of the lactoflavin or vitamin G content of the samples were made from the composite growth curves of the rats, compared with the reference curve of rats fed the standard lactoflavin, shown in the table.

Five biological estimates checked the rapid chemical determinations with a maximum difference of 25% as indicated.

Effort is being made to apply this rapid method to colostrum, shown by biological work in our laboratory to be higher in lactoflavin content than ordinary milk. Certain characteristics of the colostrum, due to physical or chemical properties, present difficulties not yet overcome.

Supplements Milk	No. rats	Daily portion, ml.	Av. gain for 8 weeks, g.	Estimated flavin per ml. milk, γ	Flavin per ml. milk Fluoray lamp, γ	Diff. from biological value per ml. γ	Diff. from biological value per ml. %
A	10	3	43	2.2	2.30	+0.10	+ 4
B	10	3	40	2.0	1.76	− .24	−12
C	10	3	46	2.3	2.20	− .10	− 5
D	10	3	54	2.6	2.55	− .05	− 2
E	10	3	50	2.5	1.88	− .62	−25
Lactoflavin		γ		Animals for reference curve			
	9	5.0	33				
None	25	..	±0	Negative control animals			

(6) Bourquin-Sherman, THIS JOURNAL, **53**, 3501 (1931).

(7) Booher, Blodgett and Page, *J. Biol. Chem.*, **107**, 599 (1934); Bisbey and Sherman, *ibid.*, **112**, 415 (1935).

KANSAS AGRICULTURAL EXPT. STA.
MANHATTAN, KANSAS RECEIVED APRIL 20, 1937

COMMUNICATIONS TO THE EDITOR

THE THERMAL DECOMPOSITION OF α-TOCOPHEROL

Sir:

Last year, H. M. Evans, O. H. Emerson and G. A. Emerson [*J. Biol. Chem.*, **113**, 319 (1936)] reported the isolation from wheat germ oil and later from cottonseed oil [*Science*, **83**, 421 (1936)] of alcohols having the biological properties of vitamin E. These substances were named "tocopherols" and appear to be isomers with the empirical formula $C_{29}H_{57}O_2$, and to be chemically closely related. Structurally, it is known that the compounds contain a hydroxyl group which accounts for one of the oxygen atoms.

I have recently investigated α-tocopherol, the most active of these substances, using material prepared from cottonseed oil, and wish to report certain observations on the behavior of this compound at higher temperatures which seem to permit deductions to be drawn regarding the constitution of α-tocopherol.

When α-tocopherol is heated decomposition sets in at 350° and a crystalline sublimate is obtained as well as an oily distillate. The crystalline material is very readily obtained in the pure state and its investigation led to a formula $C_{10}H_{14}O_2$. A literature search revealed its close resemblance to durohydroquinone and the identity was established by direct comparison with a sample of this compound kindly furnished through the courtesy of Professor Lee Irvin Smith of the University of Minnesota. Both the diacetyl derivative and the quinone were also prepared and compared by mixed melting point determinations with the corresponding derivatives of the known durohydroquinone.

ANALYSES AND MELTING POINTS

Compound		Diphenol		Diacetate	Quinone
M. p., °C.		230		201	111
Formula		$C_{10}H_{14}O_2$		$C_{14}H_{18}O_4$	$C_{10}H_{12}O_2$
Calcd.	C, %	72.28		67.18	73.14
	H, %	8.40		7.25	7.36
Found	C, %	72.29	72.32	67.12	73.42
	H, %	8.54	8.46	7.21	7.41

The above findings seem to be best explained by assigning the structure of a mono-ether of durohydroquinone to α-tocopherol. The ultraviolet spectrum of α-tocopherol is very similar to that of hydroquinone as has been pointed out to