The results obtained by varying the concentration of nickelous nitrate in the ternary solvent are presented in Table I.

Test no. 6 was made on same solution as no. 5 but with a different capillary. With a value of 13.29 g./cc. for the density of mercury at 125°, the Ilkovic equation becomes

$$i_d = 614nD^{1/2}Cm^{2/3}t_{max}^{1/6}$$

with the symbols having the usual meaning given by Kolthoff and Lingane.[2] The agreement of the experimental data with the Ilkovic equation can be seen from the essential constancy of the ratio $i_d/Cm^{2/3}t_{max}^{1/6}$ in the last column of the table. The average deviation in the ratio is ±4.3%. Substitution of the average ratio, 1.18, into the Ilkovic equation gives a diffusion coefficient equal to 9.2×10^{-7} cm.²/sec. for the nickel bearing ion.

Work is in progress to eliminate the solubility and solvent instability difficulties by employing more stable solvent electrolytes, *e. g.*, alkali halides, at higher temperatures. This will also allow investigation of a number of metals for the dropping electrode.

INSTITUTE FOR THE STUDY OF METALS
UNIVERSITY OF CHICAGO N. H. NACHTRIEB
CHICAGO, ILL. M. STEINBERG

RECEIVED MAY 7, 1948

EXCHANGE REACTIONS BETWEEN IODINE ATOMS AND ORGANIC IODIDES

Sir:

Several workers have reported exchange reactions between organic iodine compounds and inorganic iodides, but there are very few known examples of exchanges with neutral iodine atoms or molecules. Methyl iodide[1] and several diiodophenols[2] have been shown to exchange with elementary iodine in polar solvents, but the mechanisms of these reactions were not elucidated. Noyes, Dickinson and Schomaker[3] demonstrated that neutral atoms were involved in the exchange of 1,2-diiodoethylene with elementary iodine in saturated hydrocarbon solvents.

We have now observed atomic exchange reactions with some other representative organic iodides. The experiments were conducted with iodine-131 supplied by the Oak Ridge National Laboratory and obtained on allocation from the United States Atomic Energy Commission. Hexane solutions 0.002 molar (0.004 normal) in radioactive iodine and 0.04 molar in organically combined iodine were illuminated with a tungsten lamp at about 30°. The iodine in each solution was then extracted by shaking it with an acidic aqueous solution of sodium sulfite, and the activities in one or both of the separated solutions were measured with a jacketed counter. Comparative approximate rate constants based on *trans*-diiodoethylene as unity were as follows:

Allyl iodide	much greater than	200
Trans-diiodoethylene		1.0
Iodobenzene		0.002
Ethyl iodide	less than	0.001

The rate constant for allyl iodide could not be obtained with any precision, for exchange was 60% complete in twenty seconds under the normal illumination of the laboratory desk. This amount of exchange corresponds to a rate approximately 200 times as fast as the rate of exchange of *trans*-diiodoethylene under the much more intense illumination employed in the other experiments. When the laboratory was darkened to an extent such that the necessary operations could barely be carried out, exchange of allyl iodide was 25% complete in twenty seconds. Therefore, at least a large fraction of the exchange appears to involve free atoms, but the possibility of an accompanying dark reaction is not excluded. Studies of the separation procedure demonstrated that allyl iodide underwent no more than 1% of exchange with iodide ion under the conditions employed in the reduction of the iodine.

A solution of ethyl iodide which was illuminated for one week underwent a significant amount of exchange, but the data did not permit the calculation of a reliable rate constant.

That exchange in the last three compounds in the table requires free atoms is indicated by the fact that duplicate solutions stored in the dark for as much as one week underwent no more than 1% of exchange.

We are undertaking a more thorough investigation of the kinetics of these reactions.

CONTRIBUTION FROM THE CHEMICAL LABORATORIES
OF COLUMBIA UNIVERSITY
NEW YORK 27, N. Y. RICHARD M. NOYES

RECEIVED JUNE 7, 1948

AN INTERRELATIONSHIP OF THYMIDINE AND VITAMIN B_{12}

Sir:

In a series of studies on factors functionally related to folic acid and *p*-aminobenzoic acid, thymidine was isolated from liver as a factor preventing the toxicity of a competitive antagonist of folic acid.[1] The recently reported isolation of vitamin B_{12} as a growth factor for *Lactobacillus lactis* Dorner[2,3] necessitated a study of the function of the vitamin to determine whether or not it is identical with a factor found in this Laboratory to be concerned with the biosynthesis of thymidine. As vitamin B_{12} has been isolated using an assay with *Lactobacillus lactis* Dorner, this organism was utilized in the present investigation.

A medium suitable for assay techniques has not

(1) H. A. C. McKay, *Nature*, **139**, 283 (1937).

(2) W. H. Miller, G. W. Anderson, R. K. Madison and D. J. Salley, *Science*, **100**, 340 (1944).

(3) R. M. Noyes, R. G. Dickinson and V. Schomaker, THIS JOURNAL, **67**, 1319 (1945).

(1) Shive, *et al.*, THIS JOURNAL, in press.

(2) Rickes, *et al.*, *Science*, **107**, 396 (1948).

(3) Shorb, *ibid.*, **107**, 397 (1948).

been adequately described for this organism; however, a previously described medium[4] in which the phosphate buffer was replaced by sodium acetate and which was supplemented with an oleic acid source, "Tween 80," 10 mg. per 10 cc., enzymatic digest of casein,[5] 10 mg. per 10 cc., and Wilson's liver fraction LR, 100γ per 10 cc., supports good growth of the organism in the presence of liver extracts containing anti-pernicious anemia principles and can be used successfully as an assay medium. The enzymatic digest of casein replaces clarified tomato juice.[3] Tests were incubated for twenty-four hours at 37–38°.

With the above medium or one containing clarified tomato juice (0.5 cc. per 10 cc.) in place of the enzymatic digest of casein, thymidine adequately replaced the liver extracts containing anti-pernicious anemia principles. Half-maximum stimulation of growth was obtained at a concentration of 1–3 γ of thymidine per 10 cc. Thymine was inactive at concentrations as high as 100 γ per 10 cc.

When the medium containing tomato juice was utilized, as little as 1 cc. of sterile, aerated distilled water added aseptically to 10 cc. of medium replaced the liver extract, and this effect was enhanced by aseptic addition of ascorbic acid. However, when the enzymatic digest of casein was used in place of tomato juice, the aerated water was inactive, but ascorbic acid (1 mg. in 1 cc. of sterile, aerated water per 10 cc. of medium) added aseptically still adequately replaced the liver extracts containing anti-pernicious anemia principles for the nutrition of this organism. The function of ascorbic acid in replacing the liver extract will be reported separately.

Since thymidine adequately replaces vitamin B_{12} in the nutrition of *Lactobacillus lactis* Dorner, it appears probable that vitamin B_{12} functions in the biosynthesis of thymidine.

(4) Guirard, *et al.*, *Arch. Biochem.*, **9**, 361 (1946).
(5) Roberts and Snell, *J. Biol. Chem.*, **163**, 499 (1946).

THE BIOCHEMICAL INSTITUTE AND
THE DEPARTMENT OF CHEMISTRY WILLIAM SHIVE
THE UNIVERSITY OF TEXAS AND JOANNE MACOW RAVEL
THE CLAYTON FOUNDATION FOR ROBERT E. EAKIN
RESEARCH, AUSTIN, TEXAS

RECEIVED JUNE 1, 1948

A NEW COLOR TEST FOR TRYPTOPHAN

Sir:

It has been observed that at room temperature perchloric acid converts trytophan to a fluorescent yellowish green compound. Fluorescence is particularly strong in ultraviolet light. Tryptophan may readily be identified in untreated proteins by this test. This reaction is not given by other amino acids and biologic substances with which it is usually associated. Indol acetic acid, however, gives a slight pink color and slight fluorescence under the conditions of the test.

The Test.—One-half of 1 cc. of water containing 0.5 mg. of tryptophan, or about 10 mg. of albumen (egg powder) or any other tryptophan-containing protein, is placed in a test-tube. The protein does not have to be in solution. Three cc. of perchloric acid (C. P. 70–72%) is added and the contents of the tube are well mixed. A quite stable, intense greenish-yellow color develops within a few minutes attaining maximum intensity in about ten minutes. Upon the addition of 0.1 cc. of a 1% ferric chloride solution, the greenish-yellow color becomes reddish-orange. If the ferric chloride solution is added to the tryptophan-containing solution before the perchloric acid, the reddish-orange color is formed instantaneously. For the detection of minute amounts of tryptophan, ultraviolet light and perchloric acid without ferric chloride should be employed.

The following tryptophan-containing materials gave the reaction: casein, albumen (egg powder), human blood serum, pepsin and crystalline soybean trypsin inhibitor.

The following amino acids did not give the reaction: glycine, alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, cysteine, cystine, methionine, threonine, proline, hydroxyproline, histidine, arginine, lysine, serine, aspartic acid, glutamic acid, and *p*-aminobenzoic acid.

S. S. Cohen (*J. Biol. Chem.*, **156**, 691 (1944)) made the interesting observation that when carbohydrates and tryptophan were heated for ten minutes at 100° in 30% perchloric acid colored condensation products form. In Cohen's reaction boiling is an essential factor. The green fluorescent compound described in the present communication, however, forms readily at room temperature, carbohydrates do not interact and this reaction does not take place in 30% perchloric acid.

VENEREAL DISEASE RESEARCH LABORATORY
U. S. PUBLIC HEALTH SERVICE
U. S. MARINE HOSPITAL HENRY TAUBER
STATEN ISLAND 4, N, Y.

RECEIVED JUNE 7, 1948