We wish to thank Mr. E. B. Damon and Dr. W. A. Ray for the kind loan of apparatus used in this investigation.

HARVARD CHEMICAL LABORATORIES
CAMBRIDGE, MASSACHUSETTS
GEORGE DAVIS SCIENCE HALL
KNOX COLLEGE
GALESBURG, ILLINOIS RECEIVED JULY 18, 1938

Changes in the Physical Properties of Regenerated Cellulose by Liquid Ammonia

BY RICHARD G. ROBERTS

During the dialysis of proteins and hormones contained in bags made from regenerated cellulose film and placed in liquid ammonia, it was observed that the bags changed in size and in flexibility. Therefore, a series of comparative tests on some of the physical properties of the film and ammonia-treated film was made. The film used in these tests was du Pont Cellophane number 600.

The film was cut into strips of convenient size and placed in a Dewar flask. Liquid ammonia, previously dried over metallic sodium, was added to immerse the film sample completely. The film was not previously dried by us. The Dewar flask was tightly stoppered, and attached to a mercury seal. The liquid ammonia boiled off in about twenty-four hours, and any excess ammonia gas was removed by a vacuum pump.

Physical properties showing an increase:	
Tear strength (Elmendorf test)	200.0%
Tensile strength	70.6%
Thickness (flat micrometer)	152.4%
Weight (per unit area)	27.3%
Physical properties showing a decrease:	
Length (with grain)	8.3%
Width (cross grain)	17.8%
Area	24.7%

Ratios: Increase of tear strength to decrease in area, approximately 8 to 1. Increase of tensile strength to decrease in area, approximately 3 to 1.

It has been shown that bags made from regenerated cellulose film may be used conveniently for dialyzing experiments in liquid ammonia, although some space must be allowed for shrinkage.

The author wishes to thank Montgomery Ward and Company, Chicago, Illinois, for the use of apparatus in their testing laboratory.

THE DEPARTMENT OF CHEMISTRY
CHICAGO MEDICAL SCHOOL
CHICAGO, ILLINOIS RECEIVED AUGUST 29, 1938

The Specificity of the Fermentation Test for Vitamin B_1

BY ALFRED S. SCHULTZ, LAWRENCE ATKIN AND CHARLES N. FREY

The fermentation method for the determination of vitamin B_1[1-3] has been in successful operation for some time. The effect of 2-methyl-5-ethoxymethyl-6-aminopyrimidine has been described.[2] We have assayed a wide variety of substances such as non-autoclaved yeast, rice polish, vitamin pills and concentrates, solutions of crystalline vitamin, and milk, and no evidence of the interfering substance has been found. In an investigation on the metabolism of vitamin B_1,[4,5] we found reason to believe that a portion of the fermentation stimulating effect of urine is not due to vitamin B_1. While this did not appear to alter the significance of the results, it was thought very desirable to find a method for differentiating between the intact vitamin molecule and any possible breakdown product. A way of doing this has been found in the differential oxidation of the vitamin B_1 in the presence of the aminopyrimidine.

Alkaline ferricyanide in the cold will readily oxidize the vitamin to thiochrome. Preliminary experiments with a sample of thiochrome obtained from Merck and Company showed it to be inactive in the fermentation reaction. The aminopyrimidine is more resistant to oxidation and it is a simple matter to oxidize B_1 preferentially when present in addition to aminopyrimidine. A solution containing 8 gamma of the aminopyrimidine and 8 gamma of thiamin hydrochloride in a volume of 35 ml. was treated with 2.5 ml. of 1% $K_3Fe(CN)_6$ and 2.5 ml. of 50% NaOH. After standing at room temperature for five minutes the solution was neutralized with dilute sulfuric acid and made to 100 ml. A 25-ml. aliquot of this was tested in the usual manner by gas test. It gave a stimulation which corresponded exactly to 2 gamma of the aminopyrimidine (*i. e.*, the B_1 was destroyed). Parallel experiments showed that the neutralized oxidizing solution was without influence on controls with either thiamin hydrochloride or aminopyrimidine.

(1) A. S. Schultz, L. Atkin and C. N. Frey, THIS JOURNAL, **59**, 948 (1937).
(2) A. S. Schultz, L. Atkin and C. N. Frey, *ibid.*, **59**, 2457 (1937).
(3) A. S. Schultz, L. Atkin and C. N. Frey, *ibid.*, **60**, 1514 (1938).
(4) A. S. Schultz, R. F. Light and C. N. Frey, *Proc. Soc. Exptl. Biol. Med.*, **38**, 404-406 (1938).
(5) R. F. Light, A. S. Schultz, L. Atkin and L. J. Cracas, *J. Nutr.*, **16**, 333 (1938).

This technique greatly increases the specificity of the fermentation method and an investigation of the conditions necessary for its application to substances like animal tissue and excreta is under way.

THE FLEISCHMANN LABORATORIES
STANDARD BRANDS INCORPORATED
810 GRAND CONCOURSE
NEW YORK, N. Y. RECEIVED SEPTEMBER 16, 1938

COMMUNICATIONS TO THE EDITOR

CRYSTALLINE COPPER-PROTEIN POSSESSING TYROSINASE ACTIVITY

Sir:

A crystalline material has been obtained from the aqueous extract from the wild mushroom, *Lactarius piperatus*, which may be phenol oxidase, or closely related to it. The crystals were six-sided plates and undoubtedly belonged to the hexagonal system. They were insoluble in water, dilute acids and salt solutions, but soluble in an aqueous solution of secondary sodium phosphate. Analysis showed a copper content of 0.25 and 13.6% nitrogen. Their phosphate solution was active in promoting the aerobic oxidation of *p*-cresol and catechol.

The procedure followed in obtaining the crystals can be described briefly as follows. The aqueous extract of the ground mushrooms was precipitated with 0.6 saturated ammonium sulfate, redissolved in water, the latter made 0.2 saturated with ammonium sulfate and the precipitate discarded. The filtrate obtained in the last operation was reprecipitated with 0.6 saturated ammonium sulfate, the precipitate formed redissolved in water and the solution treated with three volumes of cold acetone. The precipitate thus obtained was dissolved in water and treated with alumina. The liquid separated from the alumina contained about 50% of the active oxidase. This liquid was treated with boneblack and after filtering the filtrate was again precipitated with 0.6 saturated ammonium sulfate. The precipitate from the last operation was taken up in water and had an activity of 7000 units per cc. when determined according to the Graubard and Nelson method as modified by Adams and Nelson [THIS JOURNAL, **60**, 2472 (1938)]. When this liquid was gradually acidified by acetic acid, changing the *p*H from 6.5 to 5, and allowed to stand in the ice box, crystals separated.

DEPARTMENT OF CHEMISTRY
COLUMBIA UNIVERSITY
NEW YORK CITY
HAROLD R. DALTON
J. M. NELSON

RECEIVED NOVEMBER 25, 1938

TETRAMETHYLPLATINUM AND HEXAMETHYLDIPLATINUM

Sir:

Tetramethylplatinum has been prepared in 46% yield from trimethylplatinum iodide and methylsodium. It is the most soluble organoplatinum compound so far prepared, being readily soluble in the cold in benzene, acetone, ether and petroleum ether (b. p. 60–68°). The compound crystallizes from petroleum ether as large hexagonal crystals which decompose but do not melt at elevated temperatures. *Anal.* Pt, 76.84; C, 18.32; H, 4.31. We have found that the compound is one of several by-products of the Pope and Peachey [*J. Chem. Soc.*, **95**, 571 (1909)] reaction for the preparation of trimethylplatinum iodide from platinic chloride and methylmagnesium iodide. Hydrogen chloride converts tetramethylplatinum to trimethylplatinum chloride. *Anal.* Pt, 70.20; Cl, 13.10.

Hexamethyldiplatinum has been synthesized in 60% yield by heating trimethylplatinum iodide with powdered potassium in dry benzene. *Anal.* Pt, 81.13; C, 14.55; H, 3.92. The compound is very soluble in benzene, acetone and ether, but only slightly soluble in cold petroleum ether. It is best crystallized from a benzene–petroleum ether solution. Molecular weight determinations show that hexamethyldiplatinum is not dissociated at the freezing point of benzene [mol. wt.: calcd., 480.4; found, 482]. Iodine in ether con-