monoester phosphate group in triphosphopyridine nucleotide (TPN) is esterified in the "a" position of the adenylic acid moiety of this coenzyme. The "b" nucleotidase *does not* cleave the monoester linkage of TPN. A diphosphoadenosine fragment obtained from coenzyme A, by treatment with snake venom nucleotide pyrophosphatase, was compared with the diphosphoadenosine fragment obtained from TPN under identical conditions. It was found that the fragment from coenzyme A was chromatographically different from TPN, when analysed under the same conditions and under conditions where there was no opportunity for migration of ·phosphate groupings. Treatment of the two fragments with the "b" nucleotidase yields "5" adenylic acid only with the coenzyme A product and not with the TPN derivative. This and other evidence indicates that the monoester phosphate in coenzyme A is in the "b" form, as contrasted to TPN, which is an adenylic acid "a" derivative.

We wish to thank Drs. Fritz Lipmann, G. D. Novelli, and S. P. Colowick for their interest in this work.

THE MCCOLLUM-PRATT INSTITUTE
THE JOHNS HOPKINS UNIVERSITY
BALTIMORE, MARYLAND
TE PAO WANG
LOUIS SHUSTER
NATHAN O. KAPLAN

RECEIVED MAY 1, 1952

PREPARATION OF ACETYL COENZYME A[1]

Sir:

The separation of acetyl coenzyme A (CoA) from respiring yeast and its characterization by Lynen and Reichert as a thioester[2] has been a most important contribution to the study of the acetylations and condensations in which this compound occupies the key position of being a common intermediate. It would be of considerable advantage if acetyl CoA were readily available for study in itself and as a substrate rather than an intermediate in the numerous important reactions in which it plays so prominent a role.

Stadtman has developed a method of preparing acetyl CoA enzymatically from acetylphosphate using transacetylase.[3]

This note describes a simple and efficient means of acetylating CoA from preparations of either high or low purity and containing the CoA in either the oxidized or reduced forms.

The acetylating reagent is a 5.8 M solution of sodium thiolacetate prepared from distilled thiolacetic acid and 10 M NaOH solution. The CoA is dissolved in the reagent and kept at room temperature for one hour or more. The thiolacetate is removed by adding an equivalent amount of 4 N HCl. Thiolacetic acid separates and may be almost completely removed by aeration with an aspirator for 5–10 minutes or by ether extraction. This procedure introduces an equivalent quantity of NaCl.

If the CoA is in reduced form a saturated solution of thioacetic acid may be used. This reagent is much slower but does not introduce salt.

The acetyl CoA was assayed by conversion to acetylcholine with partially purified choline acetylase from the head ganglion of Squid. A typical result is shown in Fig. 1. The experiment was performed as follows:

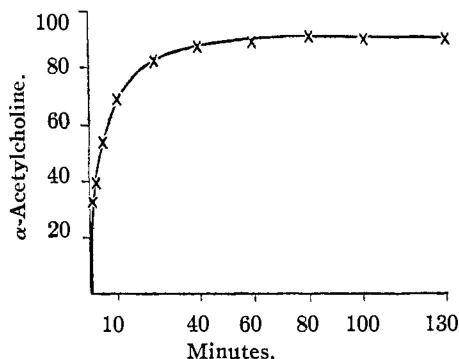

Fig. 1.

650 ug. of CoA assaying 360 units/mg. (kindly supplied and evaluated by Dr. S. Ochoa) were dissolved in 0.05 ml. of sodium thiolacetate reagent at pH about 7. After 3 hours at room temperature 0.07 ml. of 4 N HCl was added and the mixture aerated for 10 minutes. The assay was then carried out as follows: 0.5 ml. of water, 0.2 ml. of 0.7 M sodium phosphate buffer pH 7, 0.1 ml. of choline chloride (0.9 M) and $MgCl_2$ (0.01 M) solution, and finally 0.75 ml. of choline acetylase solution containing 120 μg./ml. of tetraethylpyrophosphate were added. The volume was adjusted to 2.30 ml. and samples were withdrawn at various time intervals, diluted 400 to 1200 times and tested for acetylcholine by bio-assay.[4]

No acetylcholine is obtained if enzyme, choline, or CoA is omitted or if CoA is added last after aeration.

The concentration of thiolacetate in the incubation mixture is about 3 μmol. per ml. corresponding to about 98% removal by aeration. If untreated CoA is made up as above and sodium thiolacetate is added to even 70 μmol. per ml., acetylcholine is not formed in measurable amounts. Some enzyme preparations can catalyze the formation of acetyl CoA from thiolacetate and CoA, but this squid ganglion preparation cannot.[5] These considerations show that there is no reformation of acetyl CoA during the above acetylation of choline. The method is, therefore, valid for assaying the acetyl CoA formed by the method described in this communication.

The curve shows a formation of 90 ug. of acetylcholine. Based upon the CoA assay in terms of units and assuming complete conversion to acetyl CoA and thence to acetylcholine, we should have expected 130 μg. Our yield of acetyl CoA is, therefore, at least 70%. The same yield was obtained in two cases with highly purified CoA, one in which the incubation with sodium thiolacetate extended over 1 hour and the other 3 hours. Higher yields

(1) This work was supported by a grant from the National Institute of Health, Public Health Service, National Heart Institute, Bethesda 14, Md.

(2) F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1951).

(3) Personal communication.

(4) H. C. Chang and J. H. Paddum, *J. Physiol.*, **79**, 225 (1933).

(5) D. Nachmansohn, I. B. Wilson, S. R. Korey and R. Berman, *J. Biol. Chem.*, **195**, 25 (1952)

were obtained with very impure CoA, but the CoA assay in terms of units is in this case much less reliable.

Other acetylating agents, such as acetic anhydride and isopropenyl acetate, also acetylate CoA, but the yields were markedly lower.

DEPARTMENT OF NEUROLOGY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY
NEW YORK, N. Y.
IRWIN B. WILSON

RECEIVED APRIL 9, 1952

BOOK REVIEWS

Absorptions-Spektralanalyse. By FRANZ X. MAYER, Dr.-Ing., Priv.-Doz. fur gerichtliche Chemie am Institut fur Gerichtliche Medezin der Universitate Wien and ALFRED LUSZCAK, Dr.-Ing., Mitglied des österr. Patentamtes und titl. a. o. Professor an der Technischen Hochschule Wien. Walter de Gruyter and Co., Berlin W 35, Genthiner Strasse 13, Germany. 1951. xiv + 238 pp. 14.5 × 19 cm. Price, DM 14, —.

According to the authors, this book was intended primarily for students. The main part of the text is devoted to a description of apparatus and methods for spectrophotometric analyses in the ultraviolet and visible regions of the spectrum. The authors rely only on their twenty years experience with analyses of materials bordering on chemistry and medicine, and hence do not describe recent developments in chemical spectrometry which make use of photoelectric cells and thermoelements. Eighty-five per cent. of the references are to German and French literature. Only three English texts, by Lothian, Snell and Mellon, are among the thirty-seven titles listed.

The book is divided into three divisions. In the theoretical part (60 pages) there are three chapters devoted to: A. Fundamentals of light absorption, B. Light absorption as a basis for quantitative analyses, and C. as a basis for quantitative analyses. The second division (123 pages) covers a description of: A. Condensed spark units, B. Prism spectrographs of Steinheil, Zeiss-Jena, Hilger and König-Martens, C. Cuvettes, D. Solvents, and E. Elementary photographic theory. F. Methods of measurement are described with titles such as: Hartley-Baly, Henri, Winther, Judd Lewiss Hilger, Scheibe and Pool. The three visual photometer, described in this section are the Pulfrich, Leifo and Zeiss-Ikon.

In the third division (40 pages) twelve examples of actual analyses are given with experimental details of concentrations, wave length at which absorbancies were determined and a sample calculation. Typical examples described are the determination of (1) the solubility of benzene in water, (2) benzene in ethanol, (3) strychnine and brucine in mixtures, (4) the concentration of carbon monoxide in blood, and (5) benzines in aromatics.

There are amongst the 74 figures a few typical absorption curves in which the ordinate is given in absorbancy and the abscissa in ångströms, frequencies and wave numbers.

Within the space limitations of the book, the subjects are thoroughly covered and give an excellent view of European equipment and methods. The text is free from typographical errors, it is well printed on a good quality paper. The binding conforms to the standards of good German bookbinding.

DEPARTMENT OF CHEMISTRY
PURDUE UNIVERSITY
LAFAYETTE, INDIANA
THOS. DE VRIES

Vitamins and Hormones—Advances in Research and Applications. Volume IX. By ROBERT S. HARRIS, Professor of Biochemistry of Nutrition, Massachusetts Institute of Technology, Cambridge, Mass., and KENNETH V. THIMANN, Professor of Plant Physiology, Harvard University, Cambridge, Mass. (Editors). Academic Press Inc., 125 East 23rd Street, New York 10, N. Y. 1951. xi + 395 pp. 16 × 23.5 cm. Price, $8.00.

This volume contains nine reviews; four of these are concerned with vitamins, four with hormone action, and one with vitamins and hormones.

T. H. Jukes and E. L. R. Stokstad deal in an expert fashion with the recent studies on the action of B_{12} vitamins and the folic acids. Their discussion of the effects of these vitamins on microörganisms is particularly enlightening. There is a very interesting discussion by Wm. Shive relative to the function of B vitamins in the biosynthesis of purines and pyrimidines. The author interprets many of the older results in the literature on the interrelationship of purines and pyrimidines with vitamin B_{12} and the folic acids in the light of recent discoveries linking the function of these growth factors with the metabolism of one carbon compound. The relationship between the metabolic effect of certain B vitamins, *e.g.*, folic acid and biotin, and the purines is also apparent from studies on the antimetabolites of nucleic acid metabolism which are reviewed by L. D. Wright.

The functions of biotin in enzyme systems are discussed by H. C. Lichstein. Unfortunately, up to the present date, there are very few studies with isolated enzyme systems in which a direct effect of biotin has been demonstrated. Most of the experiments reported were done with whole cells. The author suggests that the lack of correlation in biotin content and activity in the case of certain isolated enzyme systems, *e.g.*, oxalacetate decarboxylase and the malic enzyme, can be ascribed to inadequate liberation of bound biotin from the protein prior to assay. A new theory of biotin action is presented in which it is assumed that biotin exerts its effect by acting as an electron carrier.

The relationship between certain vitamins and adrenal hormones is very thoroughly reviewed by Agnes Fay Morgan. W. H. Fishman discusses the effect of estrogens on enzyme activity. A detailed account of the influence of various sex hormones on the β-glucuronidase of different tissues is given.

A brief summary of methods available for the synthesis of isotope labeled steroids is presented by G. H. Twombly. The remainder of this chapter deals with experiments on the distribution in the tissues and pathways of excretion of administered isotopically labeled steroids.

Clinical and physiological observations of the effects of cortisone and ACTH are reviewed by R. G. Sprague. The purification of urogastrone and the depression of gastric secretion by this material are discussed by M. H. F. Friedman.

INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN
MADISON 5, WISCONSIN
G. W. E. PLAUT