for the Signer sample (VII). This difference is somewhat outside probable error, and the intrinsic viscosities at 1000 sec.$^{-1}$ of 22.2 and 19.9 (100 cc./g.) support this view, but the difference is marginal in comparison with the molecular weights of DNA prepared by other methods.[1] Further evidence of the reproducibility of the DNA samples prepared by the Signer method lies in the work of Katz[3] who prepared independently another sample and using the techniques previously worked out[1] found the molecular weight to be 8,000,000.

Information on the size and shape of the DNA molecule can be derived from the angular distribution of the reduced intensity extrapolated to zero concentration. Our results show that the molecule is not at all rod-like at *p*H 6.5 but that it has a three-dimensional structure only slightly more asymmetric than a random coil. The maximum dimension of the molecule is about 6500 Å.

Two other studies indicate that the molecule is not a linear, random coil but rather a lightly branched or cross-linked polynucleotide as suggested elsewhere.[1] One of these is the investigation of the changes produced upon lowering the *p*H of the 0.2 *M* NaCl solution of DNA to 2.6 by dialysis. It is found that the molecular weight does not change but that the molecule collapses into a rod 3000 Å. long, as shown by the radical change of the angular intensity distribution to that corresponding to a rod. These results are summarized in the accompanying figure which shows the intensity distribution before, during, and after the exposure to *p*H 2.6.

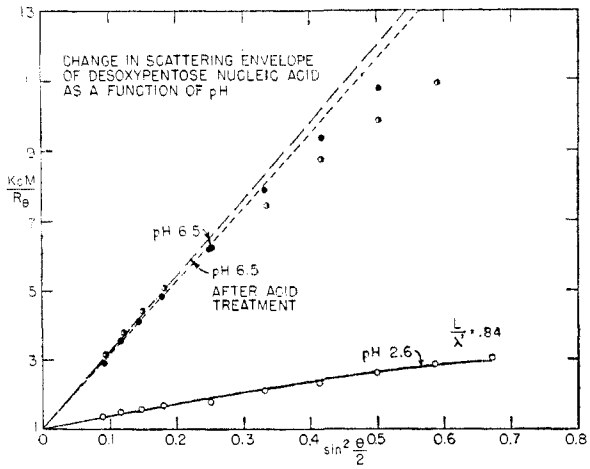

Fig. 1.

If this were a linear polynucleotide coil, it would, due to its charge, expand greatly upon removal of the salt from its solution and would exhibit an increase of the reduced specific viscosity upon dilution of its aqueous solution. In view of what has been found, for example with polymethacrylic acid,[4] this would for DNA involve about a ten-fold increase in its maximum dimension and at least a hundred-fold increase in the reduced specific viscosity. Instead it is found that at *p*H 6.5 the maximum dimension only increases about 60% when salt is removed[5] and that the reduced specific viscosity only increases from 55 at 16 mg./100 cc. to 85 at 1 mg./100 cc. Thus the polynucleotide chain is under considerable restraint probably due to a small number of branch or network points. On the other hand, even this limited expansibility of the molecule eliminates the possibility of it being rod-shaped at neutral *p*H.

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(4) A. Oth and P. Doty, *J. Phys. Chem.*, **56**, 43 (1951).

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(6) U. S. Public Health Post-doctoral Fellow.

(7) A fellowship from the American Cancer Society Institutional Grant is gratefully acknowledged.

NATURE OF MONOESTER PHOSPHATE GROUP IN COENZYME A[1]

Sir:

The coenzyme A molecule has been reported to contain three phosphate groups.[2] Two of these phosphate groups are in a pyrophosphate linkage, whereas the third phosphate has been ascertained to be in the monoester form, esterified with the ribose moiety of the adenylic acid portion of the coenzyme.[3] Prostatic phosphatase, a monoesterase, has been found to inactivate coenzyme A by removal of one phosphate.[2] We have found that an enzyme from barley,[4] which splits only "b" nucleotides, also will remove the monoester phosphate group and inactivate the coenzyme.[5] Table I shows the action of the "b" nucleotidase on the coenzyme.[6]

TABLE I

SPLITTING OF COENZYME A BY "B" NUCLEOTIDASE

	Inorg. P, μM	Arsenolysis of acetyl-PO_4 units	Acetylation of sulfanilamide units
CoA (0.05μM.)	0	14.8	14.8
CoA (0.05μM.) + "b"-Nucleotidase	0.052	0	13.9

The "b" nucleotidase has also been found to attack coenzyme A at about the same rate as it attacks adenylic acid "b," whereas the prostatic enzyme splits coenzyme A at a much slower rate than adenylic acid "b."

Kornberg and Pricer[7] have reported that the

(1) Contribution No. 28 of the McCollum-Pratt Institute. This work was supported in part by grants from the American Cancer Society as recommended by the Committee on Growth of the National Research Council, and the Williams-Waterman Fund.

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(5) Coenzyme A was assayed by its activity in the arsenolysis of acetyl phosphate by transacetylase (E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951)). Coenzyme A, inactivated by "b" nucleotidase or prostatic phosphatase, can be rephosphorylated to form active coenzyme with ATP and pigeon liver extract, so that no inactivation is apparent when the pigeon liver assay system (containing ATP, acetate and sulfanilamide) is used.

(6) The coenzyme A preparation used was approximately 90% pure (370 units per mg.), and was generously supplied by Drs. G. D. Novelli and Fritz Lipmann.

(7) A. Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.*, **186**, 557 (1950).

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.

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BALTIMORE, MARYLAND 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:

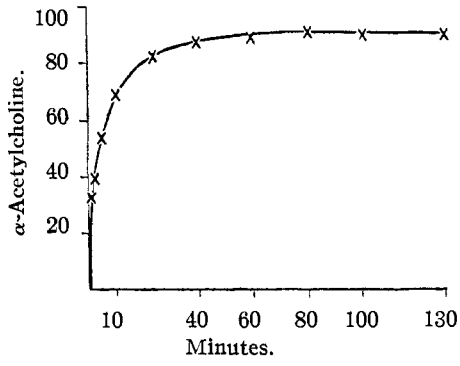

Fig. 1.

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 *p*H 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 *p*H 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.

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