We have now found that suitable adsorbents possessing anion- or cation-exchange properties permitting adsorption and elution of relatively large amounts[3] of protein under mild conditions can be prepared from α-cellulose powder. Strongly alkaline cellulose was treated with chloroacetic acid to form a cation-exchanger (CM-cellulose) or with 2-chloro-N,N-diethylethylamine to form an anion-exchanger (DEAE-Polycel).[4] These adsorbents are white or almost white, contain from 0.2–2.0 meq./g. of acidic or basic groups, and exhibit very desirable physical and mechanical characteristics.

In Fig. 1A is shown the visible banding obtained by the partial development of a CM-cellulose column with *p*H 6.5, 0.02 *M* sodium phosphate after the addition of 380 mg. of a dialyzed water extract of pig heart acetone powder. Aspartic–glutamic transaminase activity was associated with a tan band, second from the bottom. The same enzyme activity has been purified from the acetone powder 11-fold to a purity index[5] of 0.025 by chromatography with *p*H 6.75,0. 02 *M* phosphate buffer on another adsorbent prepared by precipitation of $Ca_3(PO_4)_2$ within the cellulose fiber.[6]

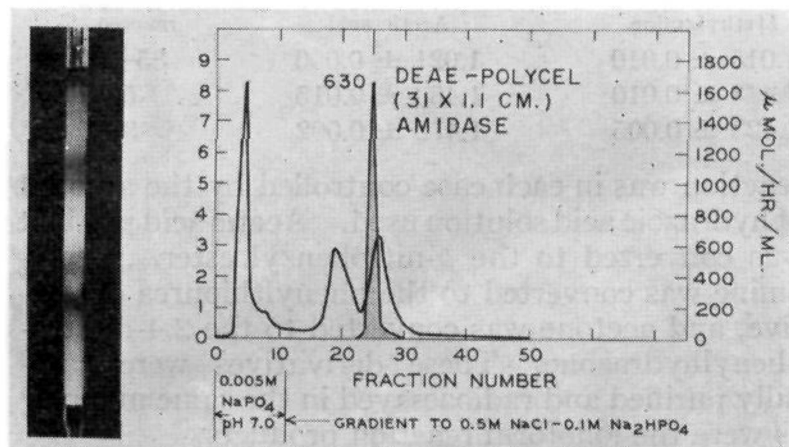

Fig. 1.—A, left: 3.5 g. CM-cellulose column, 21 × 1.1 cm. buffered initially at *p*H 5.1 with 0.02 *M* sodium phosphate; flow rate of 2 ml./hr.

B, right: 5.0 g. DEAE-cellulose buffered at *p*H 7.0 with 0.005 *M* sodium phosphate; load was 270 mg. of dialyzed, lyophilized kidney fraction in 2.7 ml. of same buffer. Fraction volume was 6 ml. Gradient was produced by continuous introduction of 0.1 *M* Na_2HPO_4–0.5 *M* NaCl into a constant volume reservoir initially containing 100 ml. of 0.005 *M* sodium phosphate, *p*H 7.0, and was begun at fraction 11. Left ordinate is optical density at 280 mμ and represents protein (solid line). Right ordinate is amidase activity and is represented by the shaded area. Specific activity of the amidase preparation was 80 μmole leucine amide split/hr./D_{280}. Fraction 25 had a specific activity of 630.

(3) The capacity of the CM-cellulose for crystalline horse carbon monoxide hemoglobin in 0.01 *M* sodium phosphate at *p*H 6.0 is about 500 mg./g. The adsorbed protein can be eluted by raising the *p*H to 7.5.

(4) We have recently become aware of the work of C. L. Hoffpauir and J. D. Guthrie (*Textile Research Journal*, **20**, 617 (1950)) who have modified cotton fabrics in a similar manner to produce anion- and cation-exchangers. F. C. McIntire and J. R. Schenk (THIS JOURNAL, **70**, 1193 (1948)) and E. B. Astwood and co-workers (*ibid.*, **73**, 2969 (1951)) have reported the cation-exchange properties of polysaccharide acid esters and of oxidized cellulose, respectively.

(5) D. E. Green, L. F. Leloir and V. Nocito, *J. Biol. Chem.*, **161**, 599 (1945).

(6) Columns of this type have been used successfully by V. E. Price and R. E. Greenfield of this Laboratory in obtaining highly active crystalline catalase from rat liver in excellent yields.

Amidase activity purified 18-fold over the original kidney homogenate by $(NH_4)_2SO_4$ fractionation was not retained by CM-cellulose buffered at *p*H 6.8. However, an additional 11-fold purification resulted because of the retardation of inactive protein. The elution diagram resulting from the application of the amidase preparation to the anion-exchanger, DEAE-Polycel, followed by development with a *p*H gradient is shown in Fig. 1B. As can be seen the shaded area containing the amidase activity was part of a larger peak of non-specific protein. A subsequent chromatogram developed with a flatter gradient, while still not providing homogeneous material, resulted in further purification and the separation of two distinct amidase activities differing in their relative rates toward leucine and alanine amides.[7]

Experiments on the anion-exchanger with highly purified calf spleen preparations containing ribonuclease, deoxyribonuclease and cathepsin have resulted in 12-fold purification of ribonuclease as well as separation from the other activities.[7] Preliminary experiments with horse serum have indicated that the resolving power of these cellulose ion-exchangers is greater than that afforded by conventional electrophoresis.

In the chromatographic fractionation of these heart, kidney and spleen enzymes, although decreased stability to dialysis and lyophilization was found, recovery of activity varied from 50–100%. Essentially quantitative nitrogen recoveries were obtained in the serum studies.

(7) The experiments with the kidney and spleen enzymes will be reported in more detail with S. M. Birnbaum and M. E. Maver, respectively.

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PARTICIPATION OF THIOCTIC ACID IN THE ACETATE-ACTIVATING REACTION[1,2]

Sir:

The well-known role of thioctic acid as an acyl carrier in the oxidation of α-keto acids[3,4] suggests that this cofactor may participate in other acyl transfer reactions. Accordingly, extracts of pigeon liver acetone powder were examined for a thioctic acid requirement in the acetate-activating reaction[5,6]

$$\text{Acetate} + \text{ATP}^7 + \text{Co A} \rightleftharpoons \text{Acetyl-Co A} + \text{AMP} + \text{PP}$$

Extracts were prepared by grinding 1 g. of powder

(1) Aided by grants from the National Institutes of Health, United States Public Health Service, from the National Vitamin Foundation, and by a contract between the Office of Naval Research and the University of Texas Medical Branch.

(2) Appreciation is expressed to Dr. Mary Ellen Jones for the courtesies extended the author during a recent visit to the Biochemical Research Laboratory, Massachusetts General Hospital, at which time several of the results reported here were reaffirmed.

(3) G. R. Seaman, *Proc. Soc. Exper. Biol. Med.*, **82**, 184 (1953).

(4) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **75**, 1261 (1953).

(5) F. Lipmann, *et al.*, *ibid.*, **74**, 2384 (1952).

(6) H. Beinert, *et al.*, *J. Biol. Chem.*, **203**, 35 (1953).

(7) The following abbreviations are used: ATP, adenosine triphosphate; Co A, coenzyme A; AMP, adenosine monophosphate; PP, pyrophosphate; TRIS, tris-(hydroxymethyl)-aminomethane.

with 10 ml. of 0.2 M $NaHCO_3$ and the insoluble material was removed by centrifuging at 2,000 × g for 30 min. Co A was removed from the supernatant by treatment with Dowex-1.[8] The crude extracts respond to the alumina procedure[3] for the removal of significant quantities of bond thioctic acid from the enzymes. Although assay[9] shows that only about 50% of the thioctic acid is removed by the alumina procedure, treated enzymes possess significantly less acetate activating activity, as measured by the hydroxamic acid method,[10] than do untreated enzymes (Table I). This indicates that although the cofactor has not been completely removed, the concentration has been decreased to below enzyme saturation level. Activity of the acetate-activating system may be restored to alumina treated preparations by the addition of 7.5 Mμg. of synthetic thioctic acid.[11]

Two fractions of the pigeon liver extracts have been separated by ammonium sulfate precipitation; the fractions were collected by centrifugation at 18,000 × g for 15 min. Fraction I (0–35% saturated) contains only very slight acetate activating activity. Fraction II (35–70% saturation) contains almost the entire activity contained in the crude extract; but it does not respond to alumina treatment. Removal of thioctic acid by the alumina procedure is achieved upon combination of the two fractions. Heating Fraction I for 10 min. at 65° completely destroys the activity. It thus appears that Fraction I functions by splitting protein-bound thioctic acid from the enzyme; liberated thioctic acid is then adsorbed and removed by the alumina.

TABLE I

Incubation mixture contained, per 1.4 ml.: 100 μM. Na acetate; 20 units Co A; 200 μM. TRIS buffer, pH 8.2; 10 μM. glutathione; 200 μM. hydroxylamine; 10 μM. Na-ATP; 10 μM. $MgCl_2$; 60 μM. NaF; enzyme solution. Incubated 90 min. at 32°. Glutathione, Na-ATP and hydroxylamine were adjusted to pH 8 with 1 M TRIS before use. Thioctic acid content of crude untreated material was 20 Mμg. per mg. protein; after alumina treatment the content was 11 Mμg. per mg. Fractions I and II contained 17 Mμg. thioctic acid per mg. protein before alumina treatment and 9 Mμg. per mg. after treatment. Alumina treatment of Fraction II alone resulted in no change in thioctic acid content.

Additions and treatment	μM. hydroxamic acid formed Crude (0.3 ml.)	Fraction I (0.3 ml.)	Fraction II (0.2 ml.)	Fractions I + II (0.1 ml.) (0.2 ml.)
None	0.96	0.02	0.52	0.54
7.5 Mμg. thioctic acid	.95	.03	.50	.53
Alumina treated	.42	..	.53	.34
Alumina treated + 7.5 Mμg. thioctic acid	.80	..	..	.47
2 μM. arsenite	.88	..	.48	.49
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite	.51	..	..	.39
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 20 μM. cysteine	.49	..	..	.40
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 20 μM. addnl. glutathione	.50	..	..	.39
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 10 μM. BAL	.84	..	..	.50

As anticipated[12] with a reaction requiring thioctic acid, the acetate-activating system is inhibited by arsenite, but not by arsenate. The arsenite sensitivity of systems containing alumina-treated enzyme plus added thioctic acid is much greater than is the sensitivity in mixtures with untreated enzymes containing an excess of the cofactor. The inhibition is reversed by BAL, but not by such monothiols as cysteine or glutathione.

(8) E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951).

(9) I. C. Gunsalus, M. I. Dolin and L. Struglia, *ibid.*, **194**, 849 (1952).

(10) T. C. Chou and F. Lipmann, *ibid.*, **196**, 89 (1952).

(11) Kindly supplied by Dr. E. L. R. Stokstad.

(12) I. C. Gunsalus, *J. Cell. Comp. Physiol.*, **41** (Suppl. I), 113 (1953).

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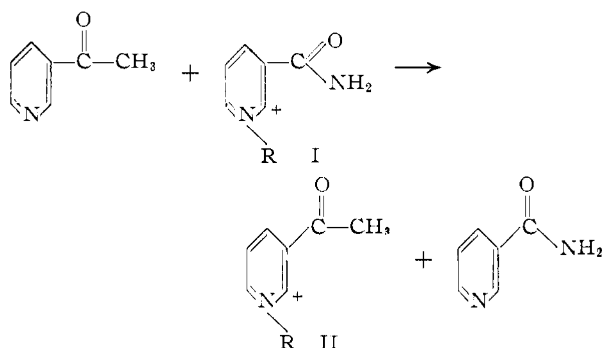
THE 3-ACETYLPYRIDINE ANALOG OF DPN[1]

Sir:

3-Acetylpyridine has been reported to produce symptoms of nicotinamide deficiency in animals but is without effect on the growth of microörganisms.[2] It has been found that the DPNase from pig brain will catalyze an exchange between isonicotinic acid hydrazide and the nicotinamide moiety of DPN (I) to form the isonicotinic acid hydrazide analog of DPN.[3] We have in a similar manner been able to demonstrate the synthesis of the 3-acetylpyridine analog of DPN (II) by the pig brain DPNase according to the equation



R represents the adenosinediphosphate ribose moiety of the DPN molecule (I). II has been isolated from a reaction mixture containing pig brain enzyme, I, and 0.1 M 3-acetylpyridine. Under these conditions, I is almost quantitatively converted to II. II was isolated by precipitation with acid acetone.

II is cleaved by the pig brain DPNase at the same rate as is I. This cleavage results in the forma-

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(2) D. W. Woolley, *Ann. N. Y. Acad. Sci.*, **52**, 1235 (1950).

(3) L. J. Zatman, N. O. Kaplan, S. P. Colowick and M. M. Ciotti, THIS JOURNAL, **75**, 3293 (1953).