TABLE I

SPECIFIC ACTIVITIES OF REACTANTS AND PRODUCTS (μc. PER MMOLE) IN THE IODOFORM REACTION

Reaction	Acetone used × 0.5	Derivative	Iodoform	Acetic acid	Derivative	Procedure
1	0.741 ± 0.004	2,4-Dinitrophenyl	0.738 ± 0.006	...	...	*a*
2	2.898 ± 0.002	Hydrazone	2.881 ± 0.007	2.863 ± 0.003	S-1-Naphthylmethylthiuronium salt	*b*
3	5.60 ± 0.03	Semicarbazone	5.57 ± 0.08	5.63 ± 0.08	*p*-Nitrobenzyl ester	*c*
4	5.60 ± 0.03	...	5.55 ± 0.06	...	...	*d*

[a] Acetone (0.200 ml.) in aqueous base was treated with excess iodine in potassium iodide solution as originally described.[1] The yield of iodoform was 80%. Acetic acid was not recovered. [b] Acetone (2.00 ml.) was added to water (200 cc.) containing iodine (14.4 g., 10% excess) and sodium iodide (20 g.); 10% sodium hydroxide solution was added dropwise until the iodine color disappeared. The iodoform was filtered, the filtrate was acidified, and sodium hydroxide was added until the solution became colorless. The additional iodoform which separated was filtered, and the process was repeated until no more iodoform separated. The total yield of iodoform was 90%. Residual iodine was destroyed with sodium bisulfite. The slightly basic filtrate was evaporated to dryness and sodium iodide was removed by acetone extraction. The acidified solution of the remaining salts was steam distilled to recover acetic acid. [c] To 2.00 ml. of acetone in 400 ml. of 2 *N* sodium hydroxide solution, 600 ml. of iodine solution (containing 15 g. of iodine and 22 g. of sodium iodide, enough for reaction of 70% of the acetone) was added. The iodoform yield was 95% based on iodine. The acetic acid was isolated in a manner similar to that described under (*b*) except that sodium acetate was extracted from the acetone-insoluble salts with hot ethanol. A 55% recovery of sodium acetate resulted. [d] Reaction was run with just enough reagent to react with only 5% of the acetone present.

TABLE II

SPECIFIC ACTIVITIES OF REACTANTS AND PRODUCTS (μc. PER MMOLE) IN THE SCHMIDT REACTION

Reaction	Acetone used × 0.5	Unreacted acetone recovered × 0.5	Methylamine	Acetic acid	Acetone reacted, %
1	1.034 ± 0.007	...	1.015 ± 0.010	1.021 ± 0.006	85–100
2	1.496 ± 0.020	...	1.443 ± 0.010	1.483 ± 0.015	50
3	1.464 ± 0.009	1.495 ± 0.002	1.423 ± 0.005	1.472 ± 0.002	30

reed electrometer for radioactivity assay. Two different samples of acetone-1-C^{14} which were used in these reactions were prepared by different synthetic methods. Both samples were carefully fractionated. One sample was subsequently distilled from calcium oxide to eliminate the possibility that it might contain acetic acid.

Table I presents the results of the four independent experiments. All samples were assayed for radioactivity using Van Slyke solution in the usual wet-combustion procedure.[4] The carbon dioxide resulting was counted in a stainless steel ion chamber. For all assays from a given reaction, the same ion chamber was used.

The data of Table I indicate that no measurable *intramolecular* or *intermolecular* isotope effect accompanies the iodoform reaction of acetone-1-C^{14}. Several sets of reaction conditions were employed including the reaction conditions originally reported.[1]

Since the Schmidt reaction of acetone-1-C^{14} has also been reported to involve a reverse *intramolecular* isotope effect,[2,3] three runs have been made in attempt to repeat this work. As Table II reveals, the data fail to confirm the reverse effect previously reported; instead, small *intramolecular* and *intermolecular* isotope effects in the usual direction are indicated. Experimental conditions used were those described in "Organic Reactions," Vol. III.[5] The reactions were carried out in benzene solution at 0 to 5° and the percentage reaction was in each case controlled by the amount of hydrazoic acid solution used. Acetic acid product was converted to the *p*-nitrobenzyl ester, methylamine was converted to the phenylthiourea derivative, and acetone was converted to the 2,4-dinitrophenylhydrazone. These derivatives were carefully purified and radioassayed in the same manner[4] as were the iodoform reaction products.

Further studies of these reactions of labeled acetone and related ketones are in progress in attempt to correlate any observed isotope fractionation factors with reaction mechanisms.

GUS A. ROPP
OAK RIDGE WILLIAM A. BONNER
NATIONAL LABORATORY MARION T. CLARK
OAK RIDGE, TENNESSEE[6] VERNON F. RAAEN

RECEIVED MARCH 1, 1954

(6) This paper is based upon work performed under Contract Number W-7405-eng-26 for the Atomic Energy Commission at Oak Ridge National Laboratory.

(4) O. K. Neville, THIS JOURNAL, **70**, 3501 (1948); V. F. Raaen and G. A. Ropp, *Anal. Chem.*, **25**, 174 (1953); for wet combustion of derivatives containing nitrogen, a lead dioxide trap at 180° was included in the line.

(5) Hans Wolff, "Organic Reactions," Vol. III, John Wiley and Sons, New York, N. Y., 1946, pp. 327–329.

CHROMATOGRAPHY OF PROTEINS ON CELLULOSE ION-EXCHANGERS

Sir:

Previous studies in this Laboratory[1] with an egg-white protein mixture and a bovine plasma albumin–hemoglobin mixture on a strong cation-exchange resin, as well as the experiments of other workers[2] with relatively stable, low molecular weight crystalline proteins on a weak cation-exchanger, were performed with commercial resins.

(1) H. A. Sober, G. Kegeles and F. J. Gutter, *Science*, **110**, 564 (1949); H. A. Sober, G. Kegeles and F. J. Gutter, THIS JOURNAL, **74**, 2734 (1952).

(2) S. Moore and W. H. Stein, *Ann. Rev. Biochem.*, **21**, 521 (1953); C. A. Zittle, *Advances in Enzymology*, **14**, 319 (1953).

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]

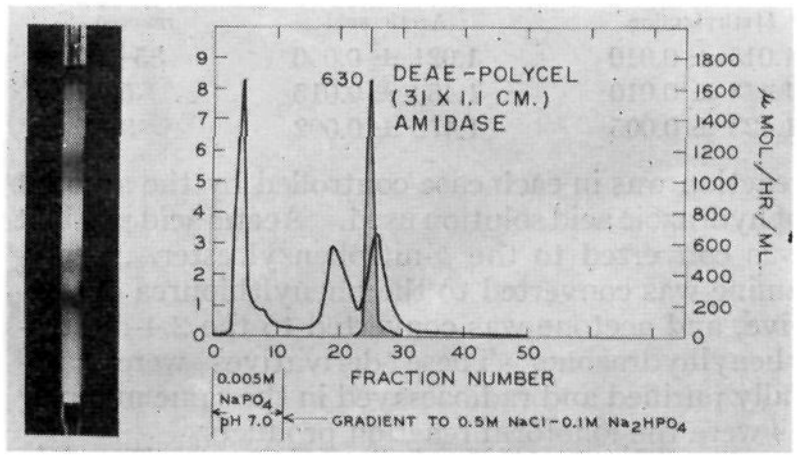

Fig. 1.—A, left: 3.5 g. CM-cellulose column, 21 × 1.1 cm. buffered initially at *p*H 5.1 with 0.02 *M* sodium phosphate; flow rate of 2 ml./hr.

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.

LABORATORY OF BIOCHEMISTRY
NATIONAL CANCER INSTITUTE HERBERT A. SOBER
NATIONAL INSTITUTES OF HEALTH ELBERT A. PETERSON
BETHESDA 14, MARYLAND

RECEIVED JANUARY 20, 1954

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.