ature independent at 3130 Å. If this compound should prove to be isopropyl iodide, a new primary process is indicated in which isopropyl radicals are formed. Further experiments are in progress to establish unequivocally the structure of this iodide and the nature of the reaction by which it is formed.

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THE ISOLATION OF AN ISOMERIC CYTIDYLIC ACID FROM HYDROLYSATES OF YEAST RIBONUCLEIC ACID[1]

Sir:

In search of an explanation for the variations in optical activity reported for different samples of cytidylic acid, from $[\alpha]_D$ + 36.7° to $[\alpha]_D$ + 49.1°,[2] we have examined various cytidylic acid fractions isolated from yeast ribonucleic acid hydrolysates. After *N* acid hydrolysis for one hour and removal of purines with silver sulfate in acid solution, the cytidylic acid fraction was separated from uridylic acid as the insoluble phospho-12-tungstate.[3] Upon recrystallization, the most insoluble fraction was converted to free cytidylic acid which, recrystallized from water, gave a product melting with decomposition at 238–239° (in bath at 230°) and giving $[\alpha]^{22}_D$ + 20.7°, *c*, 1.0 in water. *Anal.* Found: N, 12.9; P, 9.75. Calcd. for $C_9H_{14}O_8N_3P$: N, 13.0; P, 9.6. The cytidylic acid obtained from the remaining phosphotungstates gave crystalline fractions ranging in rotation from $[\alpha]_D$ + 30° to $[\alpha]_D$ + 40°, consisting evidently of nearly equimolar mixtures of the two isomers, $[\alpha]_D$ + 20.7° and $[\alpha]_D$ + 49°. The latter has been obtained from a mixture of dibrucine cytidylate and uridylate by extraction with pyridine and recrystallization of the pyridine insoluble residue from 35% alcohol. A sample with $[\alpha]^{20}_D$ + 49.4° decomposed at 233–234° (in bath at 230°). *Anal.* Found: N, 13.06; P, 9.36.

Another sample of yeast ribonucleic acid was hydrolyzed in *N* sodium hydroxide at room temperature for nineteen hours. The solution was neutralized with formic acid and the nucleotides partly removed on a Dowex-1 formate ion exchange column.[4] The cytidylic acid fraction was eluted with 0.1 *N* formic acid and again placed on a Dowex-1 formate column. Elution with 0.05 *N* formic acid gave effluents with optical density ratios, 278 mμ/260 mμ, in 0.1 *N* hydrochloric acid ranging from 1.83 to 2.0. Concentration of the fractions giving ratios from 1.83 to 1.89 to dryness and recrystallization of the residue gave a product with $[\alpha]_D$ + 20.6°, *c*, 0.5%. *Anal.* Found: C, 33.26, 33.4; H, 4.34, 4.43; N, 12.97, 13.18. Calcd. for $C_9H_{14}O_8N_3P$: C, 33.4; H, 4.03; N, 13.0. Fractions with ratios from 1.96 to 2.0 treated similarly gave a product with $[\alpha]_D$ + 49°, *c*, 0.5%. The two isomers obtained by these procedures gave similar decomposition points as found above.

The natural cytidylic acid isomer, $[\alpha]_D$ + 20.6°, agrees in properties with synthetic cytidine-2-phosphate as given by Gulland, *et al.*[5] Data on the properties of the latter compound, however, are conflicting.[6] The low-rotating compound in our experiments was not oxidized by periodate and, therefore, contained no cytidine-5-phosphate.

The cytidylic acid, $[\alpha]_D$ + 49°, on deamination[7] gave a product which could be isolated readily in 87% yield as a dibrucine salt with $[\alpha]_D$ − 58.7°, *c*, 1.0 in pyridine. The solubility of this compound and its rotation are similar to those of the dibrucine salts of both the uridylic acid usually isolated and synthetic uridine-3-phosphate.[6] The low-rotating cytidylic acid on deamination under similar conditions gave a much more soluble brucine salt which has not yet been fully characterized.

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(1) Aided by a grant from the Rockefeller Foundation.

(2) G. R. Barker, J. M. Gulland, H. Smith and J. F. Thomas, *J. Chem. Soc.*, 904 (1949); H. Bredereck and G. Richter, *Ber.*, **71**, 718 (1938).

(3) H. S. Loring, P. M. Roll and J. G. Pierce, *J. Biol. Chem.*, **174**, 729 (1948).

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(5) J. M. Gulland and H. Smith, *J. Chem. Soc.*, 1527 (1948).

(6) A. M. Michelson and A. R. Todd, *ibid.*, 2476 (1949); D. M. Brown, L. J. Haynes and A. R. Todd, *ibid.*, 408 (1950).

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(8) The report of the isolation of an isomeric cytidylic acid presented in this paper was first received on February 21, 1950.

HETEROGENEITY IN PYRIMIDINE NUCLEOTIDES

Sir:

Previous communications from this laboratory[1,2,3,4] have presented evidence for isomerism in the naturally occurring purine ribonucleotides, adenylic and guanylic acids, the nature of which remains to be established. The first step in the establishment of this heterogeneity—namely, two peaks in the ion-exchange elution diagram—has now been duplicated in the pyrimidine nucleotides, cytidylic and uridylic acids (see Fig. 1), iso-

(1) C. E. Carter and W. E. Cohn, *Federation Proc.*, **8**, 190 (1949).

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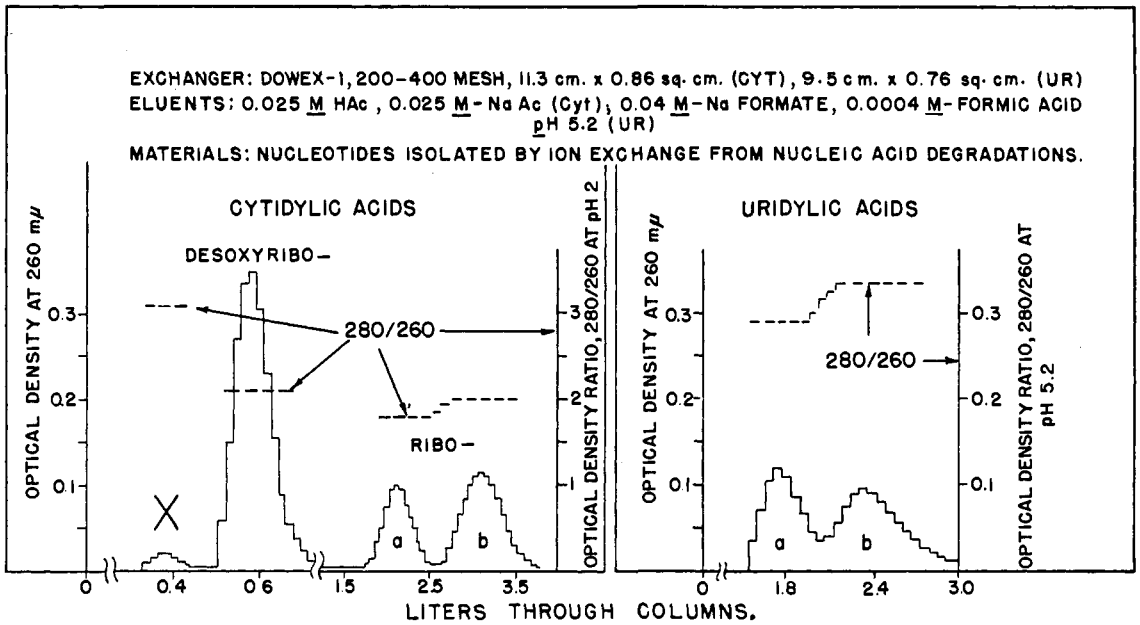

Fig. 1.—Separation of cytidylic acids and uridylic acids by ion exchange.

lated and characterized by accepted procedures,[5] in which no trace of purine compounds could be detected. This, together with small but significant differences in spectral properties, is taken as evidence of heterogeneity, if not true isomerism, in the pyrimidine nucleotides, thus raising the number of isolatable mononucleotides from yeast nucleic acid to eight. Isomerism is favored by the finding that heating for a short period in 0.1 *N* HCl is sufficient to produce an equimolecular mixture of both members of a given pair from either one.

In addition, careful analysis of the cytidylic acid fraction isolated by our usual methods[2,3,6] from an enzymatic hydrolysate of desoxyribonucleic acid[7] (which yields the four desoxyribonucleotide fractions expected) has resulted in the isolation of a small amount of material ("X" in Fig. 1) possessing spectral properties similar to, but differing significantly from, desoxycytidylic acid (280/260 *ca.* 3.1; maximum, 287 mμ at *p*H 2). The base derived from this nucleotide has an ion-exchange behavior like that of cytosine and spectral properties practically identical with those reported by Hitchings[8] for 5-methylcytosine.

Among the tentative conclusions supported by this and previously reported[2,3,4] data are that the number of constituents isolatable from nucleic acid hydrolysates may continue to increase as more refined techniques are developed and as the methods and yields of the degradation of nucleic acid (acid, alkali, enzymatic) are more carefully explored.[9]

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CONFIGURATION OF ISOMERIC ALLOTHREONINES BY ENZYMATIC RESOLUTION

Sir:

Kidney acylase and pancreatic carboxypeptidase act only on N-acylated-L-amino acids and do not affect the corresponding D-isomers. On the basis of this absolute optical enzymatic specificity, a general method of resolving the racemates of many α-amino acids has been developed.[1-5] When applied to the case of DL-allothreonine, it should be possible to uniquely and simply distinguish the configuration of each of the enantiomorphs.

DL-Allothreonine was converted to the N-chloroacetyl derivative, and recrystallized from acetone-ether; m. p. 90–92° (uncor.); N calcd. 7.2, found 7.2; yield 60%. The hydrolysis rate of the susceptible L-isomer by crude hog kidney homogenate at *p*H 7.0 and 37° is 73 μM per hour per mg. N. This rate is very nearly the same as that for the corresponding L-threonine derivative (*i. e.* 80).[1] The N-chloroacetyl-DL-allothreonine was subjected to the action of a purified hog kidney acylase, and the resolution conducted as described previously for DL-threonine and other α-

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(4) Greenstein, Gilbert and Fodor, *ibid.*, **182**, 451 (1950).
(5) Fodor, Price and Greenstein, *ibid.*, **182**, 467 (1950).