amounts of lysozyme twenty times greater than required for the elimination of activity in the absence of sucrose (Table I).

TABLE I

EFFECT OF SUCROSE ON THE INCORPORATION ACTIVITY OF LYSED CELLS

The reaction mixture contained 78 mg. of *M. lysodeikticus* cells, 86.5 μmoles of NaCl, 160 μmoles of succinate (Na) buffer (*p*H 6.5), 4.83 μmoles of carboxyl-C^{14} L-leucine[4] (5200 counts/min./μmole); sucrose and lysozyme[5] additions as indicated; final volume, 3.0 ml., incubation carried out in a Dubnoff apparatus[6] at 37° without leucine addition for 30 min.; leucine then added and mixture incubated for two hours. Reaction stopped by addition of 7.0 ml. of 10% trichloroacetic acid. Preparation of protein samples and counting procedures as previously described[7] except that nucleic acid was removed by hot trichloroacetic acid extraction.[8]

Treatment	Activity (counts/min./mg. protein)
Intact cells	29.6
200 μg. lysozyme	0.07
0.48 *M* sucrose	7.5
0.48 *M* sucrose + 200 μg lysozyme	1.5
0.48 *M* sucrose + 400 μg. lysozyme	0.9
0.48 *M* sucrose + 800 μg. lysozyme	1.0
0.64 *M* sucrose + 200 μg. lysozyme	6.1
0.64 *M* sucrose + 400 μg. lysozyme	5.3
0.64 *M* sucrose + 800 μg. lysozyme	5.6

Several lines of evidence indicate that the incorporating system is distinct from intact cells. The activity of intact cells is unaffected by repeated washing. When the sucrose-lysate was subjected to centrifugation, only the sedimentable fraction was found to have activity. Further, removing the supernatant and washing the sediment by resuspension and centrifugation markedly diminished the activity of the sediment. In one experiment the specific activity (counts/min./mg.) of the protein from the various fractions incubated separately was as follows: original sucrose-lysate, 8.2; supernatant, 0.1; sediment, 3.9; sediment washed once, 3.0; sediment washed twice, 1.4.

TABLE II

EFFECT OF DNAASE AND RNAASE ON THE ACTIVITY OF INTACT CELLS AND SUCROSE-LYSATE

Each reaction mixture contained 78 mg. *M. lysodeikticus* cells, 86.5 μmoles of NaCl, 160 μmoles of succinate (Na) buffer (*p*H 6.5), 4.83 μmoles of carboxyl-C^{14} L-leucine[4] (5200 counts/min./μmole); also where indicated 17.5 μM of $MgSO_4$, 15 μg. of DNAase,[5] 700 μg. of RNAase,[5] 0.655 g. of sucrose, 200 μg. of lysozyme[5]; final volume, 3.4 ml.; incubation, 2.0 hours after addition of leucine; preparation of protein samples as in Table I; *p*H remained constant throughout incubation.

Treatment	Activity (counts/min./mg. protein)
Intact cells	26.1
Intact cells + $MgSO_4$ + DNAase	22.3
Intact cells + RNAase	25.9
Sucrose-lysate	2.6
Sucrose-lysate + $MgSO_4$ + DNAase	11.8
Sucrose-lysate + RNAase	0.1

No intact cells were observed in the Gram-stained sucrose-lysate; nor did streaking the lysate on nutrient agar indicate the presence of viable cells. Desoxyribonuclease (DNAase) and ribonuclease (RNAase) had no effect on the activity of intact cells whereas the effect of these enzymes on the activity of the sucrose-lysate was striking. Treatment with DNAase and $MgSO_4$ increased the activity; RNAase treatment eliminated virtually all the activity (Table II).

In other experiments addition of $MgSO_4$ in the absence of DNAase resulted in only a slight increase in the sucrose–lysate activity. These enzymes did not reactivate inactive lysates produced without sucrose addition.

The production of very viscous material from the lysis of dense suspensions of *M. lysodeikticus* has been previously observed.[9] Treatment of the sucrose-lysate with DNAase produced a large drop in viscosity, indicating that the viscous material is largely desoxyribonucleic acid. Whether the drop in viscosity is related to the activating effect of this enzyme is unknown. The rate of leucine incorporation into the proteins of *M. lysodeikticus* is somewhat higher than that reported for mammalian liver cell fractions.[2] The above observations suggest that bacteria contain organized aggregates which have special properties, such as amino acid incorporation into proteins. There appears to be an analogy here to the intracellular aggregates of animal and higher plant cells.

(4) H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, *J. Biol. Chem.*, **184**, 529 (1950).

(5) Lysozyme and RNAase both crystalline products purchased from Armour Laboratories; crystalline DNAase purchased from Worthington Biochemical Sales Co.

(6) J. W. Dubnoff, *Arch. Biochem.*, **17**, 327 (1948).

(7) H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, *J. Biol. Chem.*, **196**, 669 (1952).

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URIDINE-5′-TRIPHOSPHATE

Sir:

We wish to report the isolation and identification of uridine-5′-triphosphate (UTP), the pyrimidine analog of adenosine-5′-triphosphate (ATP).

Most of the uridine-5′-nucleotides previously isolated, including those first discovered by Park and Johnson,[1] are derivatives of the 5′-diphosphate (UDP). The UDP moiety of the naturally occurring uridine nucleotides previously reported may be linked to either (1) an amino-sugar or an amino-sugar in combination with one or more amino acids, as in Park's nucleotides from penicillin-inhibited *Staphylococcus aureus*,[2,3] (2) glucose or galactose, as in cogalactowaldenase, the coenzyme of glucose-galactose interconversion[4,5] or (3) a uronic acid, as in the glucuronic acid-containing coenzyme of aminophenol conjugation.[6] The mononucleotide,

(1) J. T. Park and M. J. Johnson, *J. Biol. Chem.*, **179**, 585 (1949).

(2) J. T. Park, *ibid.*, **194**, 877, 885, 897 (1952).

(3) J. L. Strominger, *Fed. Proc.*, **12**, 277 (1953).

(4) R. Caputto, L. F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.*, **184**, 333 (1950).

(5) A. C. Paladini and L. F. Leloir, *Biochem. J.*, **51**, 426 (1952).

(6) G. J. Dutton, and I. D. E. Storey, *Biochem. J.*, **53**, XXXVII (1953).

uridine-5′-phosphate (UMP), has recently been demonstrated to be a possible intermediate in nucleic acid synthesis.[7] Enzymatic evidence for the formation of UTP was reported by Kornberg,[8] who studied the reaction of Park's UDP with phosphopyruvate, in the presence of pyruvate phosphokinase, and noted the transfer of one mole of phosphate. In fractionating ATP from rabbit muscle by means of counter-current solvent distribution, Kuby[9] noted anomalous spectral characteristics which were attributed to the presence of uridine nucleotides. Electrophoretic[10] and chromatographic[11] examination of several commercial ATP preparations had previously indicated the existence of nucleotides possessing greater mobilities and net charges than ATP. By means of ion-exchange chromatography[11] of yeast-derived nucleotides, we have isolated this electrophoretically faster fraction, purified it in gram quantities, and identified it as UTP. The purified nucleotide, isolated as a sodium salt, was found to contain about 90% UTP by electrophoretic[10] and ion-exchange[12] chromatographic analysis.

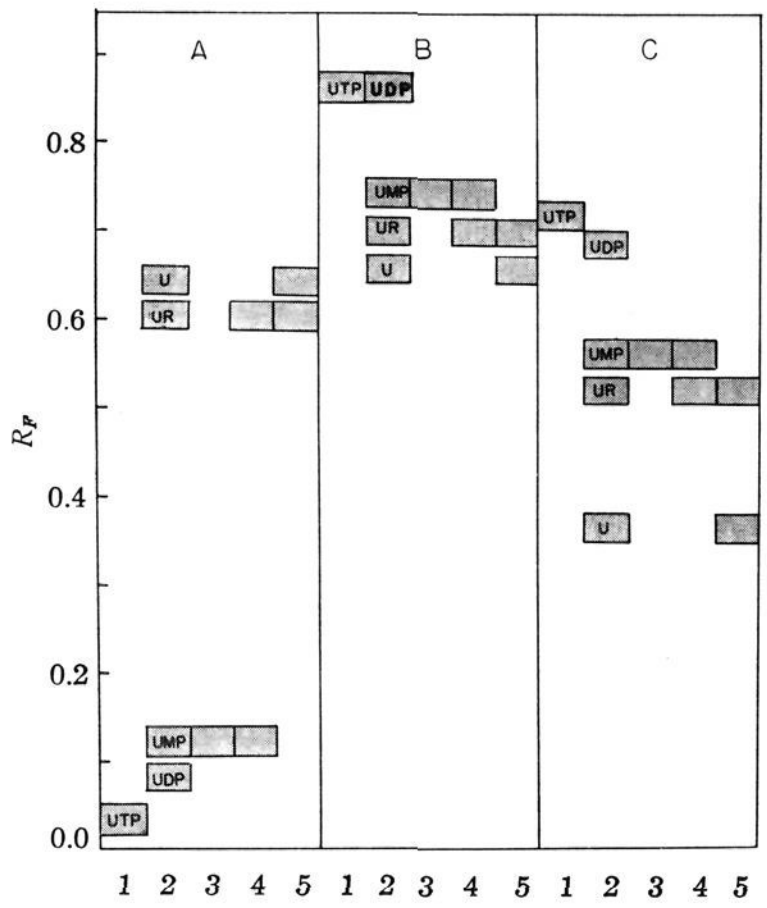

Fig. 1.—Chromatographic evidence for structure of UTP, composition of solvent systems: A, 7.5 volume ethyl alcohol + 3 volume 1 *M* ammonium acetate at *p*H 7.5; B, 5% aqueous disodium hydrogen phosphate with an overlying layer of hexyl alcohol; C, 60% ammonium sulfate + 0.1 *M* phosphate at *p*H 6.5 + 2% *n*-propyl alcohol. Paper strip number: 1, purified nucleotide designated UTP; 2, known uracil compounds (U = uracil, UR = uridine, UMP from UDP[13] by hydrolysis[5]); 3, UTP or UDP autoclaved 10 minutes in 1 *N* H_2SO_4 at 120°; 4, UTP or UDP autoclaved 2 hours in 2 *N* H_2SO_4 at 120°; 5, UTP or UDP hydrolyzed 2 hours in 90% formic acid at 175°.

The identification as a uridine derivative was based upon acid hydrolysis of the UTP under various conditions and paper chromatography of the degradation products. As shown in Fig. 1, uracil, uridine and UMP[13] were thus identified. Further confirmation of the uracil nucleus was the observed spectral shift on treatment of a solution of the UTP with bromine.[4]

Analyses for phosphorus (inorganic, total and labile) and for nitrogen and uridine were in good agreement with the UTP structure, as shown in Table I.

TABLE I

ANALYSIS OF URIDINE-5′-TRIPHOSPHATE, MONOSODIUM TETRAHYDRATE, $C_9H_{22}O_{19}N_2P_3Na$

	Calcd.	Observed
Uridine,[a] micromole per mg.	1.73	1.74
Nitrogen, %	4.85	5.00
Total phosphorus, %	16.08	15.98
Inorganic P,[b] micromoles per mg.	zero	0.06
Organic P,[c] micromoles per mg.	5.19	5.25
Labile, P,[d] micromoles per mg.	3.46	3.25
Molar ratio, P:N	1.50	1.45
Molar ratio, uridine: org. P: Labile P	1:3:2	1:3.00:1.86

[a] Using molar extinction coefficient, at 262 millimicrons, of 10,040.[14] [b] Fiske–SubbaRow method for orthophosphate. [c] Org. P = Tot. P − Inorg. P. [d] By increase in orthophosphate on hydrolysis for 15 minutes at 100° in *N* H_2SO_4.

On acid hydrolysis of the UTP preparation, no reducing sugars could be detected, indicating the absence of uridine diphosphoglucose (UDPG) and similar complex uridine nucleotides. The preparation exhibited spectral data characteristic of uridine nucleotides:

Molar Extinction Coefficient of NaH_3 UTP·$4H_2O$

Δ, mμ	*p*H 2	*p*H 7	*p*H 12
230	2100	2100	6300
240	3700	3800	5200
250	7300	7500	6200
260	9900	10100	7950
270	8700	8700	6200
280	3700	3700	2100

Electrometric titration of the isolated UTP indicates that it is most appropriately represented as the monosodium salt. The *pK* values obtained were 7.1 for the secondary phosphate and 9.7 for the enol of uracil.

Work is presently in progress to make the purified UTP available commercially for research studies.[15]

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(12) We wish to thank Dr. Robert B. Hurlbert for this analysis.

(13) We wish to thank Dr. J. T. Park for the authentic sample of UDP used in our UTP identification.

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(15) This work is being conducted at Pabst Laboratories, where the isolation studies on UTP were initially carried out.