by the unchanged nature of the "pile-up" gradient after withdrawal.

In order to determine the purity of the isolated slow sedimenting fraction, a sample of the removed solution was dialyzed against 0.15 *M* NaCl containing 0.02 *M* sodium phosphate at *p*H 7.40, and then examined in the analytical rotor. The resultant pattern, shown in Fig. 2, displayed the A component and a barely perceptible trace of the faster G component. The former exhibited a corrected sedimentation constant, $s^{\circ}_{20} = 4.09\ S$, which agrees with the literature.[6,11]

We have found this technique to be reproducible with respect to the nature of the schlieren patterns of the protein solutions obtained in the preparative tubes, the sampling of the fractions, and the analytical ultracentrifugal purity of the proteins isolated. In addition, such use of the schlieren optical system permits the determination of the location of partly sedimented protein boundaries after preparative angle ultracentrifugation without resort to chemical or biological analysis of isolated fractions. In summary, sampling controlled by observation in the schlieren optical system has a general applicability to colorless as well as colored proteins, and when applied to the slowest sedimenting component of a mixture, is capable of reproducibly yielding isolated proteins of high ultracentrifugal purity by mild physical means.

Acknowledgment.—It is a pleasure to acknowledge the asistance of Mr. Richard H. Golder and Mrs. Marilyn G. Ott in this work.

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(11) See: G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951); G. L. Miller and R. H. Golder, *Arch. Biochem. and Biophys.*, **36**, 249 (1952); J. F. Taylor, *ibid.*, **36**, 357 (1952).

COMMUNICATIONS TO THE EDITOR

THE ENZYMIC SYNTHESIS OF TREHALOSE PHOSPHATE[1]

Sir:

Uridine diphosphate glucose (UDPG)[2] has been found to disappear when incubated with a yeast extract and glucose monophosphate. This disappearance may be measured by estimating UDPG by its coenzymatic activity[3] and also as a decrease in acid-labile glucose. During the reaction UDP is formed and the reducing power of the mixture decreases. As shown in Table I, these changes are equivalent and do not take place when any one of the reactants is added at the end of the incubation period.

TABLE I

ANALYTICAL CHANGES PRODUCED BY THE ENZYME

Incubation of 0.4 μmole of glucose-6-phosphate, 0.6 μmole of UDPG and 0.02 ml. of enzyme in 0.14 *M* tris-(hydroxymethyl)-aminomethane buffer of *p*H 7 during 100 minutes at 37°; total volume, 0.1 ml.; results expressed in μmoles. The enzyme was obtained by disintegrating brewer's yeast cells with sand in a 50 cycles per second oscillator. After centrifuging the supernatant was made 0.5 saturated with ammonium sulfate and the precipitate was dialyzed.

Sample	Substance omitted during incubation[a]	Δ Reducing power[b]	Δ Labile glucose[c]	Δ UDP[d]
1	Glucose-6-phosphate	0	−0.04	+0.02
2	UDPG	0	0	0
3	None	−0.13	−0.14	+0.14

[a] The substance omitted was added at the end of the incubation period. The Δ values represent the difference with sample 2. [b] Calculated as glucose. [c] Hydrolyzed 10 minutes at pH 2 followed by precipitation with zinc sulfate and barium hydroxide. Practically all the glucose liberated under these conditions is that of UDPG. [d] Estimated by a method based on the reaction: phosphopyruvate + UDP → pyruvate + UTP (A. Kornberg, in "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951. Vol. I. p. 392). Pyruvate measured colorimetrically.

Samples equal to those shown in Table I were submitted to fractionation of the barium salts. The water-soluble, alcohol-insoluble fractions were used for paper electrophoresis with borate buffer[4] and the phosphate containing compounds were subsequently developed with a molybdate spray reagent.[5] The experiment showed that sample 3, but not samples 1 or 2, contained a phosphate compound which migrated at 60% the rate of glucose-6-phosphate. Dephosphorylation of this compound with kidney phosphatase produced a substance which gave the same R_f value as trehalose when chromatographed on paper.

In other experiments the reaction products were deproteinized by heating, treated with charcoal in order to remove the nucleotides and submitted to the action of phosphatase. When chromatographed on paper a substance migrating like trehalose was found to be present in sample 3 but not in the others. The substance extracted from the paper was hydrolyzed in 1 *N* acid during 3 hours at 100° and compared chromatographically with trehalose treated in the same manner. In both cases a glucose and a trehalose spot were obtained.

The solvent used for paper chromatography was pyridine–ethyl acetate–water[6] with which trehalose, saccharose, maltose and lactose can be separated and the developer was an alkaline silver reagent[7] which reacts slowly with non-reducing disaccharides. Furthermore, reducing from non-reducing sugars can be distinguished because only the latter give color with the aniline-phthalate spray reagent.[8] Thus the ester appears to be a phosphate of trehalose which is presumably identical to that iso-

(1) This investigation was supported in part by a research grant (G-3442) from the National Institutes of Health, U. S. Public Health Service, and by the Rockefeller Foundation.

(2) These abbreviations will be used: UDPG for uridine diphosphate glucose, UDP for uridine diphosphate, and UTP for uridine triphosphate.

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

(4) R. Consden and W. M. Stanier, *Nature*, **169**, 783 (1952).

(5) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(6) M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, **44**, 402 (1949).

(7) W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

(8) S. M. Partridge, *ibid.*, **164**, 443 (1949).

lated by Robison and Morgan[9] from the products of yeast fermentation.

The enzyme has been only partially purified and still contains the enzymes which transform glucose-6-phosphate into glucose-1-phosphate and into fructose-6-phosphate, but the most simple explanation of the chemical changes observed is the equation

$$\text{UDPG} + \text{glucose-6-phosphate} \longrightarrow \text{UDP} + \text{trehalose phosphate}$$

(9) R. Robison and W. T. J. Morgan, *Biochem. J.*, **22**, 1277 (1928).

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RECEIVED SEPTEMBER 14, 1953

PATHWAYS OF GLUCOSE CATABOLISM[1]

Sir:

The catabolism of glucose via the Embden–Meyerhof glycolytic pathway would be anticipated to result in the simultaneous contributions to carbon dioxide of carbon atoms 1 and 6 of glucose. By an alternative oxidative pathway via 6-phosphogluconate,[2] known to occur in various biological systems, the appearance of C-1 as carbon dioxide would precede that of C-6.

Glucose-1-C^{14} and glucose-6-C^{14}, the latter kindly supplied by Dr. John C. Sowden, have been compared as precursors of $C^{14}O_2$ when incubated with rat diaphragm sections, kidney slices and liver slices. The experimental conditions were identical with those described.[3] No significant differences in radiochemical yields of $C^{14}O_2$ between the two substrates was noted with diaphragm slices. The ratio

$$\frac{\text{Yield of } C^{14}O_2 \text{ from glucose-6-}C^{14}}{\text{Yield of } C^{14}O_2 \text{ from glucose-1-}C^{14}}$$

is close to unity. With kidney slices, the value of this ratio is approximately 0.9. With liver slices the mean value of this ratio is 0.36.

From studies[3] in which glucose-1-C^{14}, uniformly labeled glucose-C^{14}, lactate-1-C^{14}, lactate-2-C^{14} and lactate-3-C^{14} were compared as precursors of $C^{14}O_2$, no evidence was found supporting the occurrence of a non-glycolytic pathway in rat diaphragm sections. With kidney slices the data suggested the presence of an active non-glycolytic pathway, whereas with liver slices it appeared that the bulk of the carbon dioxide derived from glucose arose by a non-glycolytic route. A quantity, E_{max}, was defined as the maximal contribution of the glycolytic pathway to the over-all conversion of glucose to carbon dioxide. This was calculated to be 0.91, 0.72 and 0.23 for diaphragm, kidney and liver, respectively. These quantities are to be compared with the ratios obtained in the present experiments, and satisfactory agreement is to be noted.

The present experimental approach to the question of the estimation of various pathways of glucose catabolism is simpler than that previously employed and its interpretation requires fewer assumptions.

TABLE I

IN VITRO CONVERSION OF GLUCOSE-C^{14} TO $C^{14}O_2$

Tissues were incubated for 3 hours at 37.8° with 5.5 ml. of bicarbonate buffer containing 50 μM. each of glucose, gluconate, lactate and acetate. The location of the isotope in the labeled glucose is indicated below. Radiochemical yields of $C^{14}O_2$ are calculated per 500 mg. of tissue.

Tissue	Radiochemical yield of CO_2 from glucose, % -1-C^{14}	-6-C^{14}	Ratio $\frac{\text{G-6-}C^{14}}{\text{G-1-}C^{14}}$
Diaphragm	3.76	4.41	1.17
sections	3.79	3.54	0.93
	3.63	3.90	1.07
	3.89	3.56	0.92
Kidney	5.46	5.03	0.92
slices	5.38	5.02	0.93
	5.04	4.38	0.87
Liver	7.64	2.62	0.34
slices	7.19	2.46	0.34
	6.76	2.14	0.32
	10.4	3.76	0.36
	8.49	3.57	0.42

DIVISION OF NUTRITION AND PHYSIOLOGY
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NEW YORK, N. Y. — DEWITT STETTEN, JR.

RECEIVED SEPTEMBER 21, 1953

ALKALOID STUDIES. II.[1] ISOLATION OF RESERPINE AND NARCOTINE FROM *RAUWOLFIA HETEROPHYLLA* ROEM. AND SCHULT.

Sir:

Extracts of the Indian plant *Rauwolfia serpentina* Benth., characterized by an abundance of alkaloids,[2] have been used for some time in India for the treatment of hypertension and other clinical conditions.[3] Acute interest was created by the recent report[4] of the isolation from *R. serpentina* of a crystalline alkaloid, named reserpine, possessing pronounced sedative and hypotensive properties.[5] Several *R. serpentina* extracts of varying degrees of purity are already being employed clinically in this country.

At least one *Rauwolfia species*—*R. heterophylla* Roem. and Schult.—is indigenous to Central and South America and in connection with our present investigations of natural products from Latin American sources it appeared of interest to examine this plant. Such a study seemed especially pertinent because of the report[6] that the Guatemalan *R. heterophylla* ("chalchupa") contains two amorphous alkaloids—chalchupine A and B (m.p. (?) *ca.* 170 and 240°, respectively)—to which were assigned the rather implausible formulas $C_{14}H_{21}N_3O_{12}$ and $C_{15}H_{24}N_6O_{11}$. The presence of the

(1) This work was carried out while Dr. Ben Bloom held a Postdoctoral Fellowship from the Atomic Energy Commission.

(2) B. L. Horecker, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., Vol. I, (1951) p. 117.

(3) B. Bloom, M. R. Stetten and D. Stetten, Jr., *J. Biol. Chem.*, **204**, 681 (1953).

(1) Paper I, C. Djerassi, N. Frick and L. E. Geller, THIS JOURNAL, **75**, 3632 (1953).

(2) *Cf.* A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **36**, 1143 (1953), and references cited therein.

(3) *Inter al.*, M. D. Chakravarti, *Brit. Med. J.*, 1390 (1953).

(4) J. M. Müller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952). No empirical formula for reserpine was established.

(5) H. J. Bein, *ibid.*, **9**, 107 (1953).

(6) E. C. Deger, *Arch. Pharm.*, **275**, 496 (1937).

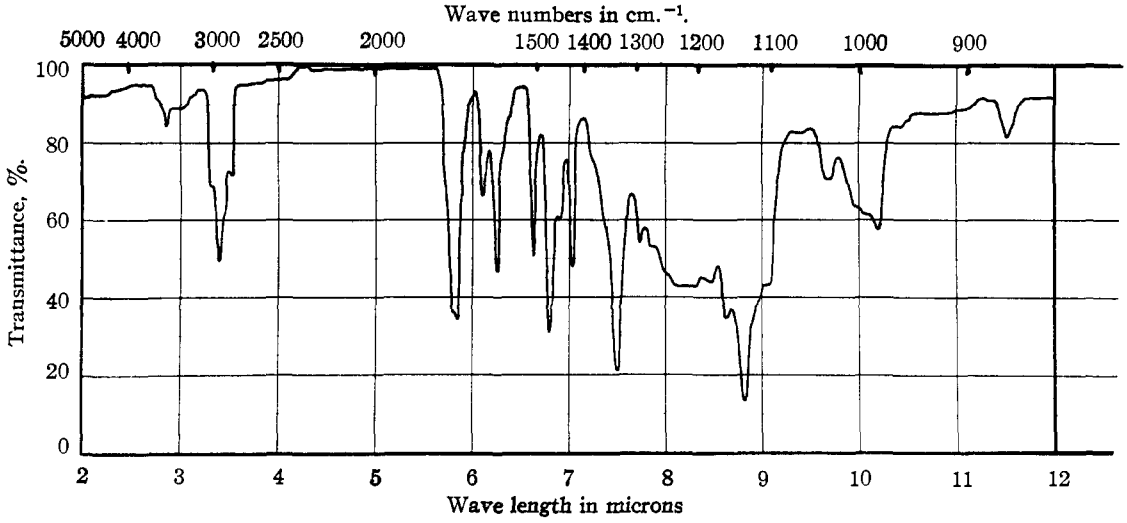

Fig. 1.—Infrared spectrum of reserpine from *Rauwolfia heterophylla* (chloroform solution, 0.1 mm. cell).

"chalchupines" has been corroborated[7] in a study of *R. heterophylla* from Colombia ("piniquepinique") and several pharmacological reports of crude extracts have appeared.[8]

Through the courtesy of Messrs. Mario and Edgar Wunderlich of Guatemala City, we have obtained some authentic *R. heterophylla* from that country while similar material from Mexico was collected by one of us near Oaxaca and identified botanically by Prof. M. Martinez. Chromatography of the benzene-soluble portion of the defatted alcoholic extract of the roots yielded two crystalline alkaloids. The earlier eluted one (m.p. 175–176°, $[\alpha]^{25}D$ −200° ($CHCl_3$), $\lambda_{max.}^{EtOH}$ 292 (3.99), 310 mμ (4.09), $\lambda_{max.}^{CHCl_3}$ 5.62 and 7.93 μ; *Anal.*[9] $C_{19}H_{14}NO_4(OCH_3)_3$ found: C, 63.93; H, 5.66; N, 3.45; methoxyl, 22.12; neut. equiv. ($HClO_4$), 402, Rast mol. wt., 420) was shown to be *l-narcotine* by direct comparison with an authentic specimen of this opium alkaloid kindly supplied by Dr. G. Moersch of Parke, Davis & Company.

The second alkaloid proved to be the widely sought-after *reserpine* (m.p. 262–263°, $[\alpha]^{25}D$ −115° ($CHCl_3$), $\lambda_{max.}^{EtOH}$ 268 mμ (4.15) shoulder at 288–297 mμ (3.95), infrared spectrum in Fig. 1) as demonstrated by direct comparison of the free base and the nitrate with material isolated from the Indian *R. serpentina* and generously furnished by Dr. M. W. Klohs of Riker Laboratories, Inc., and Dr. O. Wintersteiner of the Squibb Institute. We have been able to arrive at a satisfactory empirical formula[4,10] $C_{27}H_{22}N_2O_3(OCH_3)_6$ (Found:[9] C, 65.25; H, 6.42; N, 4.54; methoxyl, 29.83; Rast mol. wt., 619) and if it is assumed that both infrared carbonyl bands at 5.78 and 5.84 μ are due to ester groupings (one of them a methyl ester), then all nine oxygen atoms in reserpine are accounted for. Whether a biogenetic significance can be attributed to the occurrence of both narcotine and reserpine in the same plant must await the structure elucidation of the latter alkaloid. It is noteworthy that the Latin American *R. heterophylla* is the only *Rauwolfia* species other than the Indian *R. serpentina* from which reserpine has so far been isolated.

We are indebted to the Rockefeller Foundation for funds which made possible the plant collections.

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RECEIVED OCTOBER 14, 1953

(7) R. Paris and R. Mendoza D., *Bull. sci. pharmacol.*, **48**, 146 (1941).

(8) *Cf.* Raymond-Hamet, *Compt. rend.*, **209**, 384 (1939).

(9) Analyses by Mr. J. F. Alicino, Metuchen, N. J.

(10) NOTE ADDED IN PROOF.—Since submission of this paper, three pertinent articles on reserpine have appeared. Our empirical formula is in agreement with that proposed by A. Furlenmeier, *et al.* (*Experientia*, **9**, 331 (1953)) and by N. Neuss, *et al.* (THIS JOURNAL, **75**, 4879 (1953)) but not with that suggested by M. W. Klohs, *et al.* (*ibid.*, **75**, 4867 (1953)). We have confirmed the isolation of trimethylgallic acid from the saponification of reserpine as reported by these three groups.

(11) Pfizer Predoctorate Research Fellow, 1953–1954.

(12) U. S. Public Health Service Predoctorate Research Fellow, 1952–1954.

A REVERSIBLE REACTION OF BOVINE SERUM ALBUMIN

Sir:

We wish to report a reaction of bovine serum albumin (BSA) which has been discovered by a calorimetric procedure.[1] Lowering the *p*H of a BSA solution (ionic strength 0.1 *M*, chloride ion concentration 0.05 *M*) from 4.5 to 3.4 initiates a reaction which absorbs 3,100 cal. per mole of BSA at 25°. The heat absorption follows first order kinetics with high accuracy to more than 90% completion, with a half-time of approximately 2.5 min. The reaction is shown to be completely reversible by the observation that raising the *p*H from 3.4 to 4.5 results in a heat *evolution* of the same magnitude (to within 4%), also following first order kinetics with a half-time of 2.9 min. These heat effects are completely distinguished from the instantaneous heat changes which accompany changes

(1) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **73**, 2454 (1951).

in the ionization state of the protein when the *p*H is altered. The equilibrium in the reaction is dependent on the *p*H; present indications are that the equilibrium is well over on the side of higher heat content at *p*H 3.0, and on the side of lower heat content at *p*H 4.8.

The nature of the reaction is of course not shown by the calorimetric data. The fact that the kinetics of the heat changes is first order in both directions indicates that the reaction is probably not a dissociation. This conclusion is substantiated by preliminary measurements of osmotic pressures. The most reasonable possibilities would appear to be a swelling of the molecule, or an unfolding of polypeptide chains, resulting from electrostatic repulsions between the positive charges introduced by lowering the *p*H. It remains to be seen whether the reaction is related to other peculiarities in the behavior of serum albumin, such as the increase in the viscosity[2] of albumin solutions with decrease in *p*H, the decrease in sedimentation constant[3] below *p*H 4, and the electrophoretic heterogeneity[4] in the region of the isoelectric point.

An apparently similar reaction has been observed with trypsin in 0.1 *M* chloride solutions in the *p*H range 2.5 to 1.4. In this system the maximum heat change is 8,000 cal. per mole (1 mole ≈ 20,000 g.), and the reaction rates are comparable to those observed with serum albumin. Although trypsin has no enzymatic activity in this *p*H range, its activity is fully regained at neutral *p*H. Measurements of osmotic pressures indicate no change in the state of aggregation of trypsin in the *p*H range of interest. Attempts to find a reaction of similar type in the case of insulin have given negative results.

In our experiments to date we have used Armour crystallized BSA and Worthington salt-free crystalline trypsin. We plan to carry out extensive calorimetric measurements with highly purified proteins, and to parallel these measurements by detailed physical characterization in an effort to establish the nature of the reaction.

We are indebted to the Rockefeller Foundation and to the National Science Foundation for financial assistance in this investigation.

(2) S. Björnholm, E. Barbu and M. Macheboeuf, *Bull. soc. chim. biol.*, **34**, 1083 (1952).
(3) T. Svedberg and B. Sjögren, THIS JOURNAL, **52**, 2855 (1930).
(4) R. A. Alberty, *J. Phys. Coll. Chem.*, **53**, 114 (1949).

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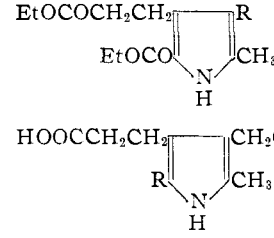
RECEIVED SEPTEMBER 19, 1953

THE SYNTHESIS OF HEMOPYRROLE-DICARBOXYLIC ACID[1]

Sir:

Of the key pyrroles related to the uroporphyrins, cryptopyrrole-dicarboxylic acid has already been synthesized.[2] We now report the synthesis of the second of these, hemopyrrole-dicarboxylic acid (IIb).

The pyrrole (Ia)[2] was converted into the glyoxylic ester (Ib), m.p. 78.5–79°, with ethyl cyanoformate and HCl. Hydrogenation in acetic acid–sulfuric acid with palladium black (ref. 3) then gave (Ic) (50%), m.p. 63–64°; *Anal.* Calcd. for $C_{17}H_{25}O_6N$: C, 60.16; H, 7.43; N, 4.13. Found: C, 59.98; H, 7.26; N, 4.26. The tricarboxylic acid (IIa), m.p. 155–156° (dec.), was obtained by alkaline hydrolysis, and partially decarboxylated with water at 100° to hemopyrrole-dicarboxylic acid (IIb), m.p. 150–150.5° (dec.); *Anal.* Calcd. for $C_{10}H_{13}O_4N$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.78; H, 6.26; N, 6.82.



(Ia) R = H
(Ib) R = COCOOEt
(Ic) R = CH_2COOEt

(IIa) R = COOH
(IIb) R = H

These structures were confirmed by decarboxylating (IIa) at 200° to hemopyrrole-carboxylic acid, m.p. 128–129° (lit. 130–131°[4]), giving the chocolate-brown methyl ester-picrate, m.p. 118.5–120° (lit. 121–122°[4]).

(3) K. Kindler, W. Metzendorf and Dschi-yin-Kwok, *Ber.*, **76**, 308 (1943).
(4) H. Fischer and H. Orth, "Chemie des Pyrrols," Akademische Verlag, Leipzig, 1934, Band I, p. 282 ff.

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RECEIVED SEPTEMBER 30, 1953

IN VITRO INCORPORATION OF LEUCINE INTO THE PROTEINS OF *MICROCOCCUS LYSODEIKTICUS*

Sir:

Lysis of *Micrococcus lysodeikticus* cells with lysozyme under certain conditions has yielded a particulate system, distinct from intact cells, which carries out the incorporation of leucine into protein. Work in this laboratory on baterial amino acid incorporation has been briefly referred to.[1,2] While this work was in progress, Gale and Folkes[3] have reported in a note similar experiments with fragmented cells of *Staphylococcus aureus*. These represent the first cases of amino acid incorporation by bacterial cell fractions.

Resting cells of *M. lysodeikticus* rapidly incorporate leucine into protein. When thoroughly washed cells are treated with lysozyme, all incorporating activity is lost. Attempts to reactivate this lysate by the addition of numerous metabolites were unsuccessful. However, the addition of sucrose during lysis resulted in maintenance of a considerable portion of the activity. The effect of the sucrose was not significantly changed by using

(1) H. Borsook, "Advances in Protein Chemistry," Academic Press, Inc., New York, N. Y., 1953.
(2) H. Borsook, *Fortschr. Chem. org. Naturstoffe*, Springer Verlag, Vienna, Austria, 1952, pp. 310–311.
(3) E. F. Gale and J. P. Folkes, *Biochem. J.*, **55**, xi (1953).

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

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,

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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(9) D. Herbert and J. Pinsent, *Biochem. J.*, **43**, 193 (1948).

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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).

(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.

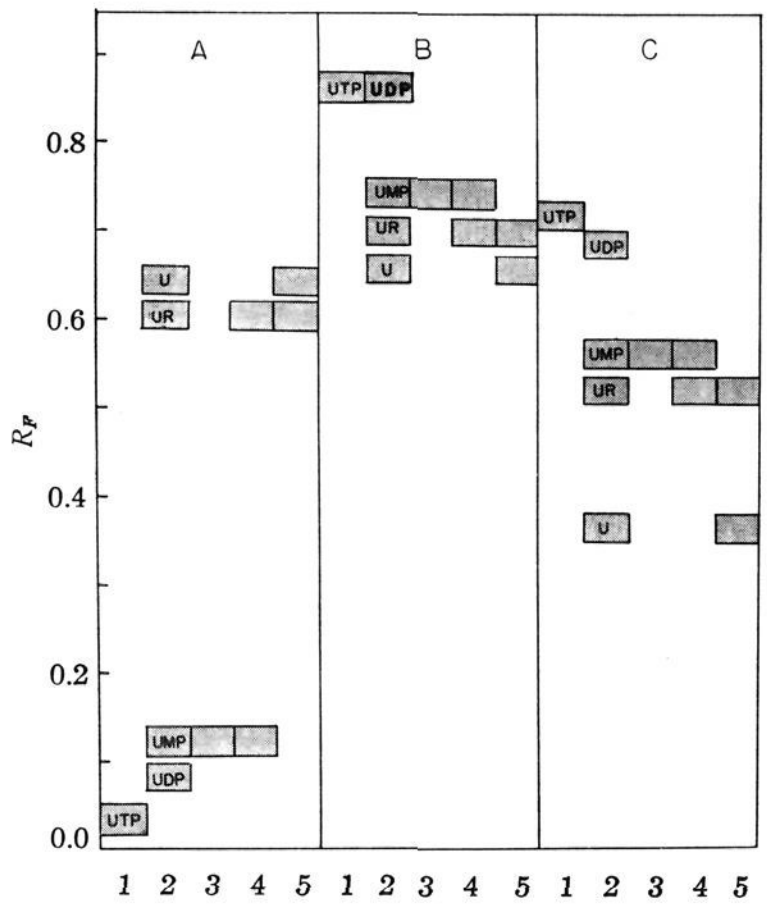

Fig. 1.—Chromatographic evidence for structure of UTP, composition of solvent systems: A, 7.5 volume ethyl alcohol + 3 volume 1 *M* ammonium acetate at *p*H 7.5; B, 5% aqueous disodium hydrogen phosphate with an overlying layer of hexyl alcohol; C, 60% ammonium sulfate + 0.1 *M* phosphate at *p*H 6.5 + 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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RECEIVED AUGUST 27, 1953

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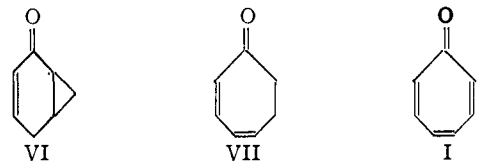
(15) This work is being conducted at Pabst Laboratories, where the isolation studies on UTP were initially carried out.

SYNTHESIS OF TROPONE *VIA* A NORCARENONE → CYCLOHEPTADIENONE REARRANGEMENT

Sir:

In view of the widespread interest in the field of tropones and tropolones, certain value should be attached to any selective synthetic route which shows promise of generality and versatility. Such a route is illustrated below by a new preparation of tropone (I) itself.[1] From the available 3,5-dihydroxybenzoic acid[2] we have obtained, by Raney nickel catalyzed hydrogenation in aqueous sodium hydroxide, 3,5-diketohexahydrobenzoic acid (II), m.p. 178.5–180° (Calcd. for $C_7H_8O_4$: C, 53.86; H, 5.16. Found: C, 53.60; H, 5.33). Treatment of the acid under esterification conditions with ethanol afforded the ethyl enol ether ethyl ester (III) of II, b.p. 125–126° at 0.4 mm., $n^{25}D$ 1.4923 (Calcd. for $C_{11}H_{16}O_4$: C, 62.25, H, 7.61. Found: C, 61.76, H, 7.36). Reduction of III with lithium aluminum hydride followed by mild acid hydrolysis[3] gave 5-hydroxymethyl-cyclohexen-2-one (IV), λ_{max} 226mμ, which was isolated and purified as the 2,4-dinitrophenylhydrazone, m.p. 147.0–148.5°, λ_{max} 252 mμ, ε 15,800:374 mμ, ε 26,600 (Calcd. for $C_{13}H_{14}N_4O_5$: C, 50.97, H, 4.61. Found: C, 50.94, H, 4.90). The *p*-toluenesulfonate, m.p. 75–75.7° (V), of IV (Calcd. for $C_{14}H_{16}O_4S$: C, 59.98, H, 5.75. Found: C, 60.21, H, 5.54), on being stirred with dilute aqueous sodium hydroxide, readily yielded, *via* norcaren-3-one (VI),[4] the rearrangement product, cycloheptadien-2,4-one (VII), λ_{max} 292 mμ, ε 5400 (characterized by catalytic hydrogenation to cycloheptanone). The ketone VII, without isolation, was converted by selenium dioxide dehydrogenation to *tropone*, which was identified by the complete agreement of its characteristic ultraviolet absorption spectrum with that already reported,[1] as well as by the preparation of two salts, the monopicrate, m.p. 99.0–100.3° (Calcd. for $C_{13}H_9N_3O_8$: C, 46.65, H, 2.84, N, 12.54. Found: C, 46.45, H, 2.98, N, 12.88) and the picrylsulfonate, m.p. 268–270°. The melting points reported for these salts are 99–100°[1a] and 266–267°,[1b] respectively. In practice one need isolate only three intermediates, II, III and V, each of which can be obtained in a yield of 60% or better.



VI VII I

In view of Doering's reported transformation[5] of tropone to 2-aminotropone (VIII) by means of hydrazine or hydroxylamine, the above approach would thus appear to embrace tropolones as well, since the parent of this class may be obtained by hydrolysis of VIII.[6] The application of this overall scheme to the synthesis of natural products containing the tropolone ring is in progress in this Laboratory.

(1) Two syntheses of tropone have been reported: (a) W. von E. Doering and F. L. Detert. THIS JOURNAL, **73**, 877 (1951); (b) H. J. Dauben, Jr., and H. J. Ringold, *ibid.*, **73**, 876 (1951).

(2) A. W. Weston and C. M. Suter, "Organic Syntheses," Vol. 21, John Wiley and Sons, Inc., New York, N.Y., 1941, p. 27.

(3) J. P. Blanchard and H. L. Goering, THIS JOURNAL, **73**, 5863 (1951).

(4) The mechanism of this rearrangement (*cf.* R. W. L. Clarke and A. Lapworth, *J. Chem. Soc.*, **97**, 11 (1910)) will constitute the subject of a separate publication.

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(6) W. von E. Doering and L. H. Knox, THIS JOURNAL, **73**, 837 (1951).

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BOOK REVIEWS

La Chimica Delle Fermentazioni. Microbiologia—Enzimologia Chimica E Tecnica Delle Fermentazioni. Second Edition. By VIRGILIO BOLCATO, Incaricato Nell' Università di Pavia. Nicola Zanichelli, Editore, Bologna, Italy. 1952. xxi + 665 pp. 18 × 25.5 cm. Price, Lire 5000 Nette.

This book is intended to bring up-to-date the first edition written in 1944–1945, which suffered from the unavailability of foreign literature. The book is written in simple Italian, so that it can be read by anyone familiar with one of the romance languages. The subject matter is presented at the level of the biochemistry graduate student. It is divided into four parts, including elements of general microbiology (89 pages), enzymology (161 pages), the chemistry of fermentations (173 pages) and the technical aspects of industrial fermentations (190 pages).

Certain sections of the book dealing with biological oxidations and chemistry of fermentations are well covered. Historical development of various major lines of work, associating names with particular contributions has also been treated well. However, the author has endeavored to cover such a very wide range of subject matter that it is inevitable that some of the material suffers from incomplete treatment. For example, the coverage of more recent literature and patents since 1945 could be better. Discussion of microbiological production of antibiotics, vitamin B_{12}, riboflavin and steroids is neglected. The book does not contain a substantial treatment of the vitamin and growth factor requirements of microörganisms, nor of the use of microörganisms for vitamin and amino acid assays. Some of the industrial fermentations described by the author might still be in use in Europe but are obsolete in this country.

The author, an authority in his field, is to be commended for having assembled such a tremendous amount of closely related material in rapidly growing fields. We believe that the book should prove of value to both the academic and technical workers in Italy, even when the subject matter is available in several fine books in the English language.

JULIUS BERGER
JOHN T. PLATI