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.

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(3) T. Svedberg and B. Sjögren, THIS JOURNAL, **52**, 2855 (1930).

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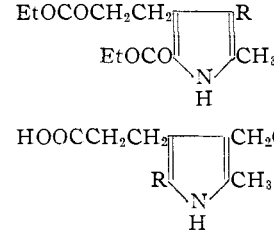
RECEIVED SEPTEMBER 19, 1953

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

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.

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

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(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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RECEIVED SEPTEMBER 14, 1953

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,

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

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

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