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.

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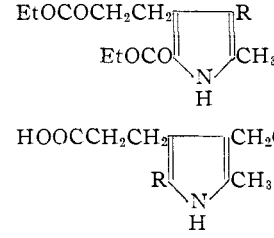
RECEIVED SEPTEMBER 19, 1953

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]).

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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

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