D-ribonate tetraacetate (m. p. 87–87.5°, $[\alpha]^{27}D$ +17° in chloroform), after removal of catalyst and solvent, was crystallized from acetone–methanol–(petroleum ether) and yielded *aldehydo*-D-ribose tetraacetate[2] in rather low yield.

Full details will be reported in a latter communication.

(2) R. Pasternack and E. V. Brown, U. S. Patent 2,237,263 (1941).

CHEMICAL LABORATORY
THE OHIO STATE UNIVERSITY M. L. WOLFROM
COLUMBUS, OHIO J. V. KARABINOS

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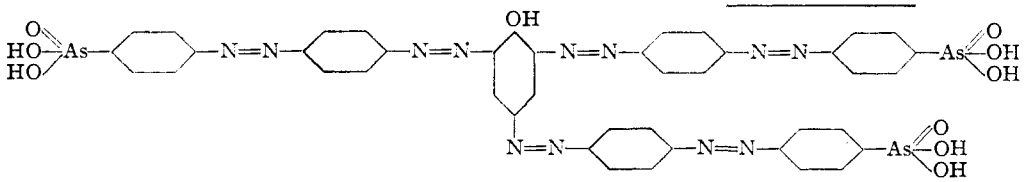
PRESSURE AND SPECIFIC PRECIPITATION

Sir:

Although the reactions that lead to specific precipitation evidently involve a combination between a di- or polyvalent antigen or hapten and specific sites on the antibody protein, probably resulting in the formation of a framework comprising large aggregates of the two species of molecules,[1] convincing evidence is lacking as to whether the protein itself becomes significantly altered in the process. The following observations bear on this question and suggest an approach for further study.

The precipitation system consisted of serum from rabbits immunized against arsanilic-azo-sheep serum and a simple trihaptenic dye antigen, *viz.*



To slow the precipitation for practical purposes, the serum was diluted 1:2, and the antigen 1:160,000. Visible precipitation occurred within a few minutes, and went to about three-fourths completion in an hour at room temperature. Small test-tubes were filled with corresponding specimens, stoppered with a rubber stopper, then placed in a water-filled steel pressure chamber, and hydrostatic pressure applied from a hydraulic pump within one to two minutes after mixing. At 10,000 pounds per square inch a scarcely visible precipitate formed during slightly more than an hour. After the pressure was released, precipitation continued in apparently the normal manner. Lower pressures were less effective, but quantitative analyses of protein nitrogen in precipitates centrifuged within less than five minutes after releasing pressure, showed decreases ranging from 55 to 77% under pressures between 1,500 and 8,000 pounds, in comparison with the amount under normal pressure. These data indicate a molecular volume increase on the order of 50 cc. or more per mole, although an accurate value requires detailed data on the rate of the reaction at different pressures.

Pressures of this magnitude oppose the reversible and irreversible denaturations of certain enzyme systems[2] and greatly retard the denaturation of purified human serum globulin at 65°.[3] These pressure effects are considerably greater than those encountered with reactions of small molecules and indicate that a number of groups are involved, or perhaps the protein molecule as a whole. They suggest that changes as extensive as those which take place in denaturation occur also in specific precipitation. An extensive quantitative study of pressure effects on the rate of specific precipitation will perhaps yield cogent data regarding the mechanism of the reaction.

THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY DAN H. CAMPBELL
CALIFORNIA INSTITUTE OF TECHNOLOGY FRANK H. JOHNSON[4]
PASADENA, CALIF.

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(1) Pauling, Campbell and Pressman, *Physiol. Rev.*, **23**, 203 (1943).

(2) Johnson, Eyring, Steblay, Chaplin, Huber and Gherardi, *J. Gen. Physiol.*, **28**, 463 (1945).

(3) Johnson and Campbell, *J. Cell. Comp. Physiol.*, **26**, 43 (1945).

(4) Guggenheim Fellow.

METABOLIC PRODUCTS OF *ASPERGILLUS USTUS*

Sir:

Publication of preliminary results by Hogeboom and Craig[1] on the application of the latter's countercurrent distribution method to the problem of isolating bacteriologically active components from the culture filtrate of *Aspergillus Ustus*[2] prompts us to report briefly the progress of our work carried out in collaboration with Mr. Joseph Kurung and Dr. Harry Bray, Superintendent of the New York State Hospital for Incipient Tuberculosis.

The crude active material,[3] obtained by ether extraction of the culture filtrate or mycelium, has been divided into a bicarbonate soluble, a carbonate soluble and a neutral fraction. By fractional crystallization three substances have been isolated from the neutral fraction in small, variable amounts, and never all three from a single sample of crude material: A.U.N.-1, m. p. 155–6°, (C, 38.24; H, 4.10; Cl, 23.87); A.U.N.-2, m. p. about 270° with severe decomposition, inadequately characterized at present; A.U.N.-3,

(1) Hogeboom and Craig, *J. Biol. Chem.*, **162**, 363 (1946).

(2) Kurung, *Science*, **102**, 11 (1945).

(3) Generously supplied to us by Mr. Joseph Kurung and the Wallerstein Company, Inc.