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).

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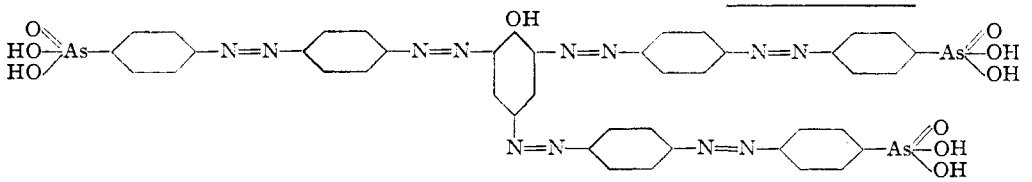
RECEIVED FEBRUARY 25, 1946

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.

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RECEIVED FEBRUARY 6, 1946

(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.

m. p. 225.5–226.5°, (C, 62.59; H, 4.81; Cl, 8.59).

The carbonate soluble fraction consistently has given the predominant active component which we are naming *ustin*. This material may be purified by fractional crystallization from toluene, acetic acid, ether, or ether–hexane, m.p. 184–186°. The probable empirical formula of ustin, $C_{19}H_{15}O_5Cl_3$, is consistent with the analytical results (C, 53.42; H, 3.69; Cl, 24.27), conductometric titration (N.E. 214), and the elementary composition of the monomethyl derivative, m. p. 174° (C, 54.09; H, 3.95; Cl, 23.54; CH_3O, 7.01), the dimethyl derivative, m. p. 147° (C, 55.00; H, 4.24; Cl, 23.19; CH_3O, 13.35), and the acetyl derivative, m. p. 212° (C, 53.68; H, 3.95; Cl, 22.76; CH_3O, 9.67). Compound I isolated by Hogeboom and Craig,[1] m. p. 185–187°, is probably identical with ustin[4] although the large discrepancy between our chlorine analysis and that obtained by Hogeboom and Craig (Cl, 22.63) suggests that their compound is impure even though isolated by the elegant countercurrent distribution method.

The antimicrobial activity of ustin, A.U.N.-1, and A.U.N.-2 are indistinguishable by any of the *in vitro* tests used so far. In synthetic media, the compounds inhibit the growth of Gram positive cocci and mycobacteria (including pathogenic tubercle bacilli) to the same degree, but are inactive against Gram negative bacilli. Their antiseptic activity increases rapidly with the H^+ concentration of the environment, but is sharply inhibited by the addition of serum albumin, and of certain other organic substances (lipids for example) to synthetic media. Thus any one of the compounds can exert an inhibitory effect on the growth of tubercle bacilli, streptococci or staphylococci in a dilution of 1/500,000 at *p*H 6.5 in a simple synthetic medium, but will fail to retard growth even in 1/50,000 in more complex media at slightly alkaline reactions.

We wish to express our sincere appreciation to Dr. Adalbert Elek for the excellence of his microanalytical assistance.

The details of this work will be published shortly.

(4) Dr. Craig very kindly examined the melting points and found no depression (m. p. and mixed m. p. 186–188°).

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RECEIVED MARCH 8, 1946

SURFACE FILM THICKNESSES OF POLYMERIC SUBSTANCES IN RELATION TO MOLECULAR STRUCTURE[1]

Sir:

It is generally well known that certain compounds, such as long chain fatty acids, can form monomolecular films on water and that the areas of such films can be related to the dimensions of the molecules.[2] Certain polymeric substances including cellulose derivatives,[3] proteins[4] and the polymer of ω-hydroxydecanoic acid[5] likewise form stable films whose areas can be conveniently measured.[6] At this time we wish to report that we have prepared films of some rubber-like polymers as well and that the results appear to give useful information about their molecular structures, particularly in relation to branching.

Five ml. of a very dilute benzene solution of a polymer ($c = 2 \times 10^{-6}$ g./ml.) is distributed over the water surface of a hydrophil balance. After the benzene has evaporated, the film area is measured in the usual manner.[7] Low conversion samples of GR-A (butadiene-acrylonitrile copolymer) gave films about 6 Å. thick whereas high conversion samples exhibited thicknesses as great as 30 Å. It is our belief that a large film thickness indicates a high degree of branching and that the corresponding polymer molecules can be better represented as "bottle brushes" than as straight chains. Other polymers containing hydriphilic groups have also been investigated; these include copolymers of butadiene with methacrylonitrile, 1-cyanobutadiene, *m*-fluorostyrene and some vinylpyridines.

Stable films for polymers such as natural rubber or GR-S could not be obtained directly. However, these substances can be investigated by the film technique if they are first rendered hydrophilic by the addition of thiocyanogen to some of the double bonds according to the methods of Pummerer and Stark and of Rehner.[8] After using their procedures, we found natural rubber and gutta percha to form relatively thin films (about 8 Å. thick) whereas the film thicknesses of GR-S exhibited wide variations with polymer conversion, indicating once more marked differences in branching.

On the whole, the method appears to show considerable promise as a tool for learning more about the structure of polymers.

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RECEIVED FEBRUARY 18, 1946

(1) The work reported in this letter was done in connection with the Government research program on synthetic rubber under contract with the Office of Rubber Reserve, Reconstruction Finance Corporation (results first reported in November, 1945).

(2) Harkins, Davis and Clark, THIS JOURNAL, **39**, 541 (1917); Langmuir, *ibid.*, **39**, 1848 (1917).

(3) Katz and Samwel, *Ann.*, **472**, 241; **474**, 296 (1929); Adam, *Trans. Faraday Soc.*, **29**, 90 (1933); Harding and Adam, *ibid.*, **29**, 837 (1933).

(4) Hughes and Rideal, *Proc. Roy. Soc.* (London), **A137**, 62 (1932).

(5) Harkins, Ries and Carman, *J. Chem. Phys.*, **3**, 692 (1935).

(6) The authors are indebted to Dr. Anderson Pace, Rayon Department, E. I. du Pont de Nemours and Co., Buffalo 7, N. Y., for calling to their attention the possibility of such measurements.

(7) See for example, Adam, "The Physics and Chemistry of Surfaces," Oxford University Press, London, 1941, 3rd ed., p. 28.

(8) Pummerer and Stark, *Ber.*, **64**, 825 (1931); Rehner, *Ind. Eng. Chem.*, **36**, 118 (1944).