Excellent growth was obtained if such a defined medium[2] was supplemented with yeast extract, peptone, or a crude biotin concentrate. These crude products could not be replaced by riboflavin, nicotinic acid, *p*-aminobenzoic acid, inositol, pimelic acid, adenine, guanine, uracil, yeast nucleic acid, oleic acid, glucosamine, asparagine, serine, choline, or folic acid.[3]

A preparation of the growth factor was obtained from yeast extract by precipitation with lead hydroxide, after first removing much inactive material with lead acetate at *p*H 4.0. The lead hydroxide precipitate was fractionally decomposed by first removing color and other impurities with alcoholic 0.1 *N* hydrochloric acid. The lead was then removed by decomposing the residue with hydrogen sulfide. The resulting solution was deanionized with Amberlite IR-4, and was then treated with Norite. The colorless and active solution so obtained was free of thiamin, nicotinic acid, pyridoxine, folic acid, biotin, *p*-aminobenzoic acid, and riboflavin. The recovery was 2–10%. The actual degree of purification was over 200-fold, but this is in all probability a low value since Amberlite IR-4 contributes considerable solids to the final preparation.

The growth factor may be partially adsorbed on the cation-exchange resin Amberlite IR-100 and on Decalso (at *p*H 0.5, but not at *p*H 3.0). Little or no adsorption on Norite (*p*H 3.0 to 7.5), Amberlite IR-4 or Permutite (*p*H 7.0) was observed. The active principle was not precipitated by uranium, silver, barium (water or 70% ethanol), phosphotungstic acid or 95% ethanol. Both mercuric salts and picric acid precipitated the growth factor, while lead only did so from crude extracts. Extraction by ether from acid, alkaline or neutral solution was not obtained. The above properties indicate that the growth factor is not identical with either sporogenes vitamin[4] or with strepogenin.[5]

Inactivation experiments have provided some data on the chemical behavior of the growth factor. Treatment in absolute ethanol with excess anhydrous hydrochloric acid leads to inactivation, while the activity may be regenerated by mild alkaline hydrolysis. Nitrous acid completely inactivates the substance, whereas oxidation with potassium permanganate at *p*H 7.5 does not affect it. While stable to normal acid or alkali at room temperature, complete inactivation occurs in five minutes at 100°. No labile phosphate is present in the best preparations. More complete elucidation of the chemical properties must await the isolation of the growth factor in pure form. With this in view large scale preparations are now in progress.

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(2) Besides the described growth factor, salts, amino acids and glucose, the organism requires biotin, pantothenic acid, thiamin and pyridoxine for growth.

(3) Kindly supplied by Dr. Roger Williams.

(4) Pappenheimer, *Biochem. J.*, **29**, 2057 (1935).

(5) Woolley and Sprince, reported before the Biochemical Division of The American Chemical Society, New York, N. Y., September 14, 1944.

DEPENDENCE OF POLYMER PROPERTIES ON TEMPERATURE OF POLYMERIZATION

Sir:

In a recent paper on polystyrene, Alfrey, Bartovics and Mark[1] showed that the values of certain characteristic constants (μ, a, K and k') deduced from osmotic pressure and viscosity data, although practically independent of molecular weight, are quite dependent on the temperature at which the polymerization occurred. This unexpected result they interpreted, properly, as indicating "a different internal architecture of the macromolecules" in the different samples produced at different temperatures. They suggested that different amounts of branching of the polymer chains in the different samples might account for the observed results.

Against the branching hypothesis it may be pointed out that more branching would be expected in a polymer produced at a high temperature than in one produced at a low temperature, that on the average (for the same molecular weight) the high-temperature polymer molecules would therefore have a less extended form than the low-temperature polymer molecules, and that the former would therefore be characterized by a smaller value of the exponent a in the equation relating viscosity to molecular weight. Actually, the reverse is the case.

An alternative explanation seems to me more reasonable. At each CHR group in a polymer chain there are two different, non-equivalent dispositions of the H and the R relative to the plane containing the C–C bonds which join this group to the adjacent C atoms in the chain. Once a chain is formed, the disposition of H and R relative to this plane cannot be changed, without a reaction equivalent to a Walden inversion; mere rotation around single bonds will not do it. In a polymer produced at a low temperature one would expect a tendency toward some *regular* sequence of dispositions of H and R, such as one in which all of the R groups would be on the same side of the plane of the zigzag carbon chain if the molecule were stretched out, or one in which the R groups alternated from one side to the other. In a polymer produced at a high temperature, however, a more *random* sequence would be expected. Difference in randomness of these dispositions would produce differences in the average degree of coiling of the chains (hence in a and K), in the solute–solvent and solute–solute interac-

(1) T. Alfrey, A. Bartovics and H. Mark, THIS JOURNAL, **65**, 2319 (1943).

tions (hence in μ), and in the frictional forces between segments of solute molecules and the surrounding solvent (hence in k'). The directions and magnitudes of these effects, however, are difficult to predict without further information.

One may predict similar effects for other high polymers of the general formula $(CH_2CHR)_n$, but not for polyethylene nor for polymers having the general formula $(CH_2CR_2)_n$, with all R groups alike. Experiments are in progress here to test this prediction.

It may be noted that randomness of the H and R dispositions relative to the chain bonds is probably also responsible (in part) for the general poor degree of crystallinity of stretched $(CH_2CHR)_n$ polymers, as shown by X-ray diffraction data.[2]

(2) M. L. Huggins, Paper presented at the Rochester meeting of the American Physical Society, June 24, 1944.

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ROCHESTER, NEW YORK MAURICE L. HUGGINS

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STOICHIOMETRIC COMPLEXES OF SERUM ALBUMIN AND SODIUM DODECYL SULFATE

Sir:

Electrophoretic and chemical analyses indicate that complex formation between crystalline horse serum albumin (A) and purified sodium dodecyl sulfate (D) is due to stoichiometric combination.

In phosphate buffer, *p*H 6.8, $\mu = 0.2$, three electrophoretic components, A, AD_n, and AD_{2n} have been identified by their respective mobilities, 4.9, 7.8, and 9.8 $\times 10^{-5}$ cm.^{2} sec.$^{-1}$ volt^{-1}. Either or both complexes may be present in solution, their proportion depending on the protein–detergent weight ratio. The electrophoretic composition of AD_n and AD_{2n} corresponds, respectively, to the maximal and minimal weight ratios requisite for complete precipitation of the protein at *p*H 4.5.[1]

Likewise, mixtures corresponding to these ratios are essentially homogeneous in electrophoresis. This agreement suggests that between *p*H 4.5 and 6.8, combination is independent of *p*H and involves protein groups, presumably cationic, whose state of ionization does not change within that range. The amount of D bound in AD_n (0.22 g. per g. protein, *i. e.*, $n = 55$ moles D per mole A) corresponds approximately to one-half the acid binding capacity of A while that bound in AD_{2n} (0.42–0.45 g.) is equivalent to the total acid binding capacity.

On both sides of the isoelectric point, low *molar* concentrations of D cause a large increase in the *relative* viscosity of serum albumin.[2] However, up to a protein-detergent weight ratio of one, at *p*H 6.8, the increase of the *intrinsic* viscosity (η_{sp}/c) depends only on the weight ratio. In this region, with decreasing A/D ratios, η_{sp}/c first remains nearly constant because the intrinsic viscosity of AD_n is comparable to that of A (4.3 *vs.* 4.1). Thereafter, η_{sp}/c increases because the intrinsic viscosity of AD_{2n} is higher (6.1). A further increase results from more extensive unfolding of the complex.

AD_n probably consists of albumin covered with a single hydrophobic layer of detergent anions bound to cationic groups. It is unlikely that AD_{2n} results from non-polar adsorption of a second layer of detergent to AD_n, with hydrophilic groups exposed, because: (1) both AD_n and AD_{2n} are insoluble below *p*H 4.8; (2) AD_{2n} but not AD_n exhibits a large viscosity increase over A. The formation of AD_{2n} is tentatively ascribed to the partial unfolding of AD_n with liberation of additional cationic groups hitherto accessible to hydrogen ions but not to large detergent anions. The viscosity increase observed with additional detergent indicates further unfolding either with: (a) formation of a third postulated complex, AD_{4n}, representing non-polar adsorption of D by AD_{2n}, or (b) by non-stoichiometric association of detergent molecules with all available groups.

Quantitative studies of complex formation with detergent mixtures are complicated by the dependence on chain length of the equilibrium between free and combined detergent.

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(1) F. W. Putnam and H. Neurath, THIS JOURNAL, **66**, 692 (1944).

(2) F. W. Putnam and H. Neurath, paper presented before the Division of Physical and Inorganic Chemistry at the 107th Meeting of the American Chemical Society at Cleveland, Ohio, April 4, 1944.