reaction. Reasonable yields were obtained only if the reaction was carried out in a very small volume.

In a typical experiment 50 mg. of adenosine-5′-phosphoric acid, 0.02 ml. of concentrated H_2SO_4 and 240 mg. of dicyclohexylcarbodiimide were shaken vigorously at room temperature in 0.05 ml. of pyridine + 0.03 ml. of water. After 5 and 10 hours, 250 mg. of carbodiimide in 0.15 ml. of pyridine was added. After 15 hours, ice-cold water was added, the *p*H was adjusted to 3 and the solution was filtered and extracted several times with ether. The solution was then subjected to electrophoresis on a cellulose column[7] (length = 40 cm., diam. = 3 cm., 4/cm. for 18 hours; 0.1 *M* ammonium formate, *p*H 3). After elution the different compounds were localized by radioactivity and ultraviolet absorption (Fig. 2).

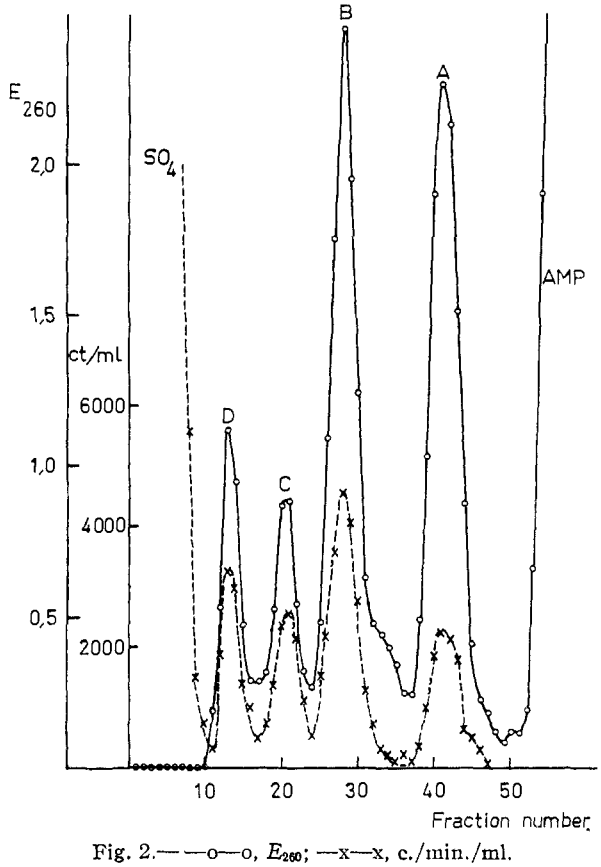

Fig. 2.— —o—o, E_{260}; —x—x, c./min./ml.

The compounds corresponding to peaks A-C all contained adenine, ribose and phosphate in the approximate ratio 1:1:1. For every molecule of adenine, A contained one molecule of sulfate, B contained two molecules of sulfate and C contained three molecules of sulfate.

Compound A could be separated into two components during electrophoresis at *p*H 8.7, both of which contained one sulfate group per molecule of AMP. The slower moving component (A_1) was identified as adenosine-5′-phosphosulfate and the faster moving component (A_2) as a mixture of adenosine-5′-phosphate-2′-sulfate and adenosine-5′-phosphate-3′-sulfate (Fig. 1; S_3 = H; S_1 or S_2 = SO_3H, S_2 or S_1 = H) by the following criteria.

(1) The sulfate group was removed completely from A_1 by treatment at 100° either with 0.5 *N* perchloric acid for 5 minutes or with calcium oxide[8] for 30 minutes, while by the same treatments only *ca.* 20 and 10% free sulfate was produced from A_2.

(2) Compound A gave rise on deamination with HNO_2 to a component which consisted of inosine-5′-phosphate and sulfate in stoichiometric amounts. This demonstrates that neither A_1 nor A_2 contains sulfuric acid bound to the amino group of adenine.

(3) One mole of A_1 consumed one mole of periodate[9] at room temperature during a period of three hours, while one mole of A consumed less than 0.1 mole of periodate. Very little free sulfate was liberated during this treatment. This demonstrates the presence of two hydroxyl groups attached to adjacent carbon atoms in A_1.

(4) The electrophoretic behavior at *p*H 8.7: adenosine-5′-phosphosulfate (A_1) contains at this *p*H two negative charges and moves slower toward the anode than adenosine-5′-phosphate-2′(or 3′)-sulfate (A_2), which contains three negative charges (Fig. 1: S_3 = H).

(5) A study of the time course of the synthesis revealed that A_1 was the dominating primary product. This is in accordance with what is known about the general mechanism of the carbodiimide method.

(6) On paper chromatography in Na_2HPO_4: isoamyl alcohol A_2 separated equally in amount into two components. This medium was originally used for the separation of adenosine-2′-phosphate and adenosine-3′-phosphate.[10]

Compound B did not consume periodate and was partially hydrolyzed by acid and alkali. We believe this disulfate to be a mixture of three compounds (Fig. 1; two of S_1, S_2 or S_3 = SO_3H, the third one = H).

The criteria for compound C indicate that it contains sulfate groups linked to both ribose hydroxyls and to the phosphate group (Fig. 1; S_1, S_2 and S_3 = SO_3H).

The identity of compound D is not yet clear.

The relative proportions of the different adenosine-5′-phosphosulfates could to some extent be modified at will by choosing the proper experimental conditions. It has thus been possible to obtain a 40–50% conversion of AMP to adenosine-5′-phosphosulfate on a one gram scale.

(7) J. Porath, *Biochem. Biophys. Acta*, **22**, 151 (1956).
(8) K. Lohmann, *Biochem. Z.*, **233**, 460 (1931).
(9) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).
(10) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

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MOLECULAR WEIGHTS OF "LIVING" POLYMERS*

Sir:

The polymerization initiated by negative aromatic ions, like naphthalene$^-$, and carried out in non-proton donating solvents like tetrahydro-

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furan leads to "living" polymers[1,2] which are capable of growing if a monomer is available. Such polymers can be eventually "killed" by adding, *e.g.*, a drop of water which transfers a proton to the active carbanion ends and turns them into inactive alkyl groups. It was suggested that the initiation by negative hydrocarbon ions results from an electron transfer to the monomer,[1,2] and eventually the dimerization of the primarily formed species produces polymers with both ends "living."

These assumptions lead to the conclusion that the number average molecular weight of the living polymers should be simply determined by the ratio Monomer/($^1/_2$ Catalyst), and should be independent of the concentration of monomer or catalyst and of the temperature of polymerization. Moreover, if some conditions are fulfilled, the resulting polymers should be mono-dispersed, *i.e.*, the number average molecular weight $\bar{M}_n$ should be identical with the weight number average molecular weight $\bar{M}_w$. The latter conclusion was confirmed by the recent measurements of Prof. G. V. Schulz, Mainz, Germany, who found the ratio $\bar{M}_w/\bar{M}_n$ for three samples prepared by us to be 1.06, 1.12 and 1.06.

The results reported here prove that the molecular weights of the living polymers are independent of the concentration (Table I) and of temperature (Table II and graph I). It is impossible to overstress the caution and care which should be taken in performing such experiments. Minute amounts of impurities left in the monomer or the solvent, or adsorbed on the walls of the reaction vessel can ruin the quantitative aspect of the investigation. The experimental difficulties rise sharply with the increasing molecular weight of the sample prepared, and thus the scatter of the results increases accordingly. This is shown clearly in Fig. 1, the points are clustering at low values of Monomer/($^1/_2$ Catalyst), but are spread more and more apart as the ratio $M/(^1/_2\ C)$ increases. Nevertheless, it is clear that a linear relation exists between $\log(\eta)$ and $\log\{M/(^1/_2\ C)\}$ proving that the relation Degree of Polymerization = Monomer/($^1/_2$ Catalyst) holds indeed, and that it is independent of temperature for the wide range -80 to 0°. Using the least square method we determined the relation $\bar{M}_w = \bar{M}_n = 141{,}000\ [\eta]^{1.35}$ or $[\eta] = 1.6 \times 10^{-4} \times \bar{M}^{0.74}$. The relation found by Schultz[3] is $[\eta] = 0.64 \times 10^{-4} \times \bar{M}^{0.75}$. The agreement in the exponent is remarkable; however, no explanation is offered for the difference in the pre-exponential factor.

TABLE I

POLYMERIZATION OF STYRENE; KILLED WITH WATER

T°, C.	Styrene/ Tetrahydrofuran	M/(1/2 C)	[η]
0	1:4	467	.472
0	1:8	480	.473
0	1:16	467	.436

TABLE II

POLYMERIZATION OF STYRENE; KILLED WITH WATER

M/(1/2 C)	[η]	M/(1/2 C)	[η]
Temp., 0°		Temp., −20 to 40°	
125	0.179	389	0.372
130	.176	484	.603
132	.165	522	.586
201	.245	Temp., *ca.* −60°	
272	.247	113	0.160
285	.311	127	.150–.180
289	.274	219	.250
336	.423	250	.270
400	.397	540	.400
467	.472	562	.450
467	.436	730	.710
480	.473	1800	1.200
565	.657	Temp., −78°	
721	.621	317	0.324
833	.577	631	.630
1190	.701	463	.591
		881	.582
		1090	.802
		1115	.994

(1) M. Szwarc, M. Levy and R. Milkovitch, THIS JOURNAL, **78**, 2656 (1956).

(2) M. Szwarc, *Nature*, **178**, 1168 (1956).

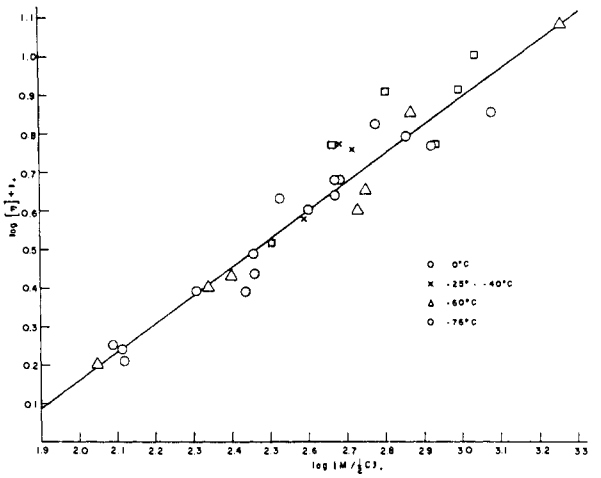

Fig. 1.—Polymerization of styrene: ○, 0°; x, −25 to −40°; △, −60°; □, −78°. $M/^1/_2C$ denotes the degree of polymerization over $^1/_2$ catalyst.

(3) G. V. Schulz, *Z. Elektrochem.*, **60**, 199 (1956).

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FOLIC ACID REDUCTASE

Sir:

The suggestion[1] that folic acid derivatives may function in oxidation–reduction systems is supported by the observation that partially purified extracts of *Clostridium sticklandii*[2] catalyze the reduction of folic acid (FA) and teropterin (diglutamyl FA) to dihydrofolic acid (FAH_2) and dihydroteropterin. This reduction requires substrate levels of CoA, and an electron donor[4] such as pyruvate, serine, α-ketobuterate or methionine. FAH_2 formation is associated with a stoichio-

(1) B. L. O'Dell, J. M. Vandenbelt, E. S. Bloom and J. J. Pfiffner, THIS JOURNAL, **69**, 250 (1947).

(2) Formerly named *Clostridium HF*, and used in studies of glycine formation from serine.[3]

(3) B. E. Wright, *J. Biol. Chem.*, **219**, 873 (1956).

(4) TPNH or DPNH—generating systems do not replace the electron donors, nor do they further reduce FAH_2 to tetrahydrofolic acid (FAH_4).