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)	$[\eta]$
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)	$[\eta]$	M/(1/2 C)	$[\eta]$
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).

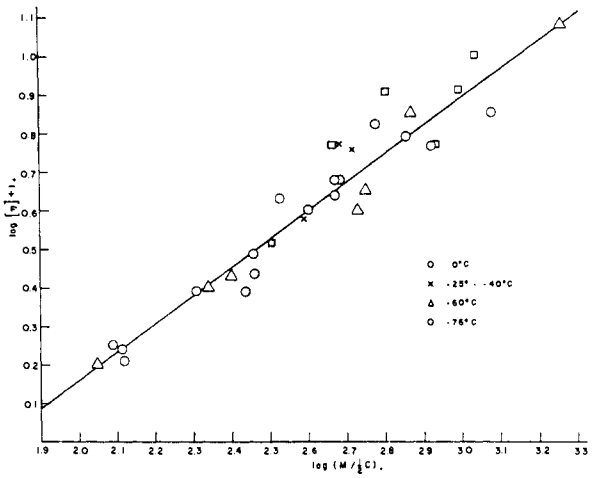


Fig. 1.—Polymerization of styrene: ○, 0°; x, −25 to −40°; △, −60°; □, −78°. $M/^1/_2C$ denotes the degree of polymerization over $^1/_2$ catalyst.

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

DEPARTMENT OF CHEMISTRY R. WAACK
NEW YORK STATE COLLEGE OF FORESTRY A. REMBAUM
AT SYRACUSE UNIVERSITY J. D. COOMBES
SYRACUSE 10, NEW YORK M. SZWARC

RECEIVED FEBRUARY 20, 1957

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).

metric increase in the amount of acetyl S CoA and CO_2 (see Table I), suggesting the reaction: FA + pyruvate + CoASH → FAH_2 + AcSCoA + CO_2. However, since pyruvate is also decomposed by non-pterine-dependent reactions, a further purification of the enzyme system is necessary before complete stoichiometry can be determined.

TABLE I

DIHYDROPTERINE FORMATION

Conditions: 1-C^{14}-pyruvate, 2.0 μM; CoA, 1.6 μM; K-succinate, *p*H 6.2, 20.0 μM in a total volume of 0.4 ml., incubated anaerobically 1 hr., 38°. Exp. I: 0.67 μM FA and 2.0 mg. protein; Exp. II: 0.5 μM teropterin and 1.6 mg. protein.

	Experiment I			Experiment II		
Omission	FAH_2[a]	AcSCoA[b]	CO_2[c]	$TerH_2$[a]	AcSCoA[b]	CO_2[c]
None	0.51	1.10	1.32	0.15	0.53	0.55
Pterine	.00	.67	1.00	.00	.38	.42
CoA	.00	.00	.12	.00	.00	.006
Pyruvate	.00	.00	.00	.00	.00	.00

[a] Calculated by assay (a). See text. [b] Determined by hydroxamic method.[5] [c] Trapped as $K_2C^{14}O_3$ and converted to $BaC^{14}O_3$.

A complete enzymatic conversion of FA to FAH_2 is shown in the accompanying figure. FAH_2 can be assayed spectrophotometrically by (a) the decrease in optical density at 365 mμ at *p*H 11, (b) the increase in optical density at 420 mμ

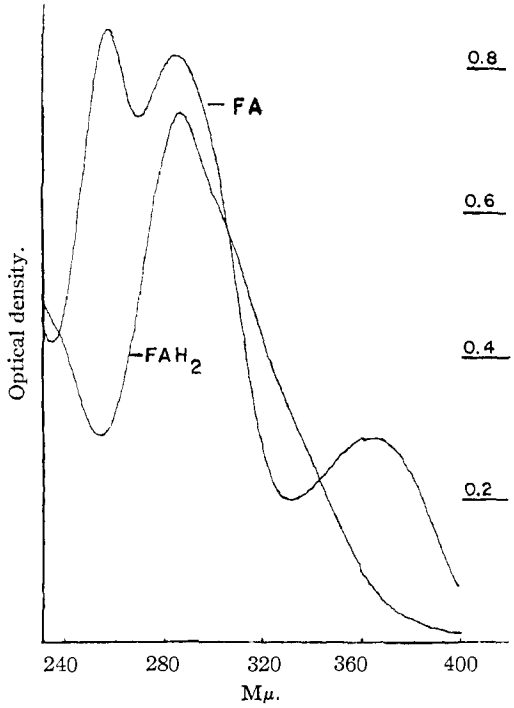


Fig. 1.—FAH_2, complete system; FA, serine omitted. The molecular extinction of FA and FAH_2, respectively, at 284 mμ, *p*H 11, is 25,200 and 22,600. Each sample was run at *p*H 11 against an enzyme blank in the reference cuvette; a Cary recording spectrophotometer was used.

following acidification, due to the formation of a yellow degradation product. Identification of FAH_2 rests on the following criteria: (I) the spectrum exhibits the expected maximum at 284 mμ, minimum at 253 mμ, and 280/340 ratio of 3.0; conversion of FA to FAH_2 also results in isosbestic points similar to those of O'Dell, *et al.*[1] (II) comparison with synthetic FAH_2,[1] which is enzymatically stable in the system, showed the same acid degradation product (assay (b)), both with respect to the time required for its optimal formation as well as the concentration of this product, as judged by the optical density at 420 mμ. (III) synthetic FAH_2 and the enzymatic product were compared chromatographically, as were their respective yellow degradation products, and found to exhibit similar R_f values in three solvent systems. FAH_4 does not fulfill criteria I, II or III. Aminopterin does not inhibit the reduction of FA to FAH_2.

(5) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **158**, 505 (1945).

SECTION ON ENZYMES OF THE LABORATORY OF CELLULAR PHYSIOLOGY AND METABOLISM
NATIONAL HEART INSTITUTE BARBARA E. WRIGHT
NATIONAL INSTITUTES OF HEALTH MINNIE L. ANDERSON
BETHESDA 14, MARYLAND

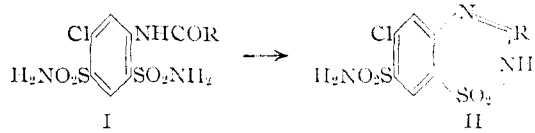
RECEIVED FEBRUARY 20, 1957

BENZOTHIADIAZINE DIOXIDES AS NOVEL DIURETICS

Sir:

Organic mercurials and carbonic anhydrase inhibitors have proven clinically useful as diuretic agents,[1] but both have certain undesirable properties. The toxicity and poor oral absorption of organic mercurials and the tendency to create electrolyte imbalance, the early and frequent development of refractoriness and the low level of potency of carbonic anhydrase inhibitors have proved serious limitations in the treatment of conditions associated with fluid and electrolyte retention. This communication concerns a new type of highly active compounds that are orally effective and possess the favorable biological properties common to both classes of the forementioned drugs.

While studying aromatic sulfonamides in our laboratories, an unexpected high order of activity has been observed with benzenedisulfonamides, particularly with the benzene-1,3-disulfonamides. Certain substituents on the benzene ring augment the activity; chlorine, amino, or acylamino have marked enhancing effects. However, further study of the chemistry of compounds of this type (I) wherein an acylamino group occupies a position *ortho* to one of the sulfamyl groups has led to novel compounds of still greater interest. Ring closure



occurs between the sulfamyl group and the adjacent acylamino group to yield a benzothiadiazine dioxide (II). Cyclization has been accomplished with a variety of acyl derivatives, both aliphatic and aromatic, including a series of homologous acyl compounds (I) where the acyl group ranges from formyl (R = H) to hexanoyl (R = C_5H_{11}). Ring closure is especially facile in the formyl derivatives and the resulting benzothiadiazines are of particular biological interest.[2]

(1) *Cf.* R. W. Berliner and J. Orloff, *Pharmacol. Rev.*, **8**, 137 (1956).

(2) We are indebted to Drs. K. H. Beyer, J. E. Baer and their associates for the biological data.