TABLE I

PROPERTIES OF THE 3-INDOLEMETHYLSULFUR COMPOUNDS

No.	Compound	Formula	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	3-Indolemethyl methyl sulfide	$C_{10}H_{11}NS$	87–88	67.74	67.38	6.25	5.90
2	3-Indolemethyl ethyl sulfide	$C_{11}H_{13}NS$	48–49	69.07	69.20	6.85	6.52
3	3-Indolemethyl *n*-propyl sulfide	$C_{12}H_{15}NS$	47	70.19	69.9	7.36	7.52
4	3-Indolemethyl *n*-butyl sulfide	$C_{13}H_{17}NS$	43–44	71.50	71.48	7.81	7.81
5	3-Indolemethyl *n*-amyl sulfide	$C_{14}H_{19}NS$	47–48	72.10	72.11	8.21	8.30
6	3,3′-Diindolemethyl sulfide	$C_{18}H_{16}N_2S$	140–141	73.94	73.49	5.52	5.63
7	S-(3-Indolemethyl)-thioglycolic acid	$C_{11}H_{11}NSO_2$	110–111	59.71	60.00	5.01	4.72
8	3-Indolemethyl methyl sulfone	$C_{10}H_{11}NO_2S$	154–155	57.41	57.55	5.30	5.01
9	3-Indolemethyl ethyl sulfone	$C_{11}H_{13}NO_2S$	142–144	59.17	59.74	5.87	5.87
10	3-Indolemethyl phenyl sulfone	$C_{15}H_{13}NO_2S$	160–161 dec.	66.40	66.72	4.83	4.98
11	3-Indolemethyl *p*-toluenesulfone	$C_{16}H_{15}NO_2S$	162 dec.	67.35	67.37	5.30	5.13
12	N-(3-Indolemethyl)-benzenesulfonamide	$C_{15}H_{14}N_2SO_2$	160–163	62.91	63.22	4.91	4.84

reduction of the sulfonyl chloride in ether by Zn or Na_2SO_3[3] and used either as the free acid or the Zn or Na salts. The following preparations are submitted as representative.

3-Indolemethyl *n*-Amyl Sulfide.—Two grams of gramine (0.0114 mole), 0.5 g. of NaOH and 1.2 g. (0.0114 mole) of *n*-amyl mercaptan were refluxed for 15 min. in 25 cc. of H_2O, cooled, acidified with dilute acetic acid to remove unreacted gramine. The oil which at first forms solidifies on cooling and scratching. The solid after slurrying in H_2O is recrystallized from pet. ether (30–50° fraction).

S-(3-Indolemethyl)-thioglycolic Acid.—3.4 g. of gramine, 2.0 cc. of thioglycolic acid, 25 cc. of 1 *N* NaOH and 25 cc. of H_2O were refluxed for 30 min. At the end of 15 min. all solid went into soln. Upon cooling and acidifying with dilute acetic acid a solid separated which was washed with H_2O and recrystallized from benzene.

3-Indolemethyl Methyl Sulfone.—Two grams (0.0114 mole) of gramine and 2 g. of zinc methyl sulfinate (0.009 mole) in 40 cc. of 95% C_2H_5OH were refluxed for 1 hr. The contents became milky white. The vapor gave a basic test to litmus and the strong odor of dimethylamine was detected. The contents was filtered after cooling to remove small amounts of inorganic zinc salt. The alcohol solution was evaporated almost to dryness, water added, and the whole extracted with ether. The ether extract upon evaporation yielded a solid which was slurried in dilute acetic acid and then H_2O; recrystallized from EtOH.

N-(3-Indolemethyl)-benzenesulfonamide.—Gramine (1 equiv.) and benzenesulfonamide (2 equiv.) were refluxed in water for 20 min. at which time an oil formed which solidified on cooling. The solid was washed with dil. HOAc, H_2O, EtOH, and ether, m.p. 160–163° (from benzene).

3,3′-Diindolemethyl Sulfide. (A).—One gram of gramine (0.0057 mole) and 0.72 g. of $Na_2S\cdot 7H_2O$ (0.003 mole) in 20 cc. of H_2O were refluxed for 30 min. and filtered hot. The residue was slurried twice with hot H_2O and recrystallized from CH_3OH to give white crystals, m.p. 140–141°.

(B).—Reaction with $NaSH\cdot 3H_2O$ gave a white solid, m.p. 142°, mixed melting point with A was 142°.

(3) F. Ullmann, *ibid.*, **34**, 1153 (1901).

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Lack of Inhibition of Desoxyribonuclease by Heat Depolymerized Desoxyribonucleic Acid[1]

BY N. B. KURNICK[2]

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In a recent report,[3] we noted that desoxyribonuclease (DNase) depolymerized highly polymerized desoxyribonucleic acid (DNA) at a much greater rate than it did heat depolymerized DNA. In order to determine whether the slower depolymerization of heat treated DNA in our experiments was due to inhibition of the enzyme by the products of heat treatment or only to lesser affinity of the DNase for the heated DNA, the following simple experiment was performed.

Method.—A sample of the substrate solution (*p*H 7.5) for DNase determination by our methyl green method[4,5] was heated for 30 minutes in a boiling water-bath. After cooling, the rates of depolymerization of heated DNA, untreated substrate, and equal parts of the heated and unheated substrates were determined.[5] The enzymes used were crystalline bovine pancreatic DNase and rabbit serum.

Results.—The results are presented in Tables I and II. Typical experiments are shown in Fig. 1. Comparison of the slopes of curves 1 (unheated DNA) and 2 (heated DNA) confirms our earlier finding that heated DNA is depolymerized at a much lower rate than is the unheated substrate. Since the slopes of curves 1 and 3 (mixture of equal parts heated and unheated DNA) are nearly equal, it follows that there is no inhibition by heated DNA. In none of the 9 experiments with crystalline DNase

TABLE I

VELOCITIES OF DEPOLYMERIZATION OF HEATED AND UNHEATED DNA BY CRYSTALLINE DNASE

Date	DNase, μg./19 ml. substrate–enzyme mixture	Slopes[a] Unheated (P) v_1	Heated (H) v_2	Mixture (P) + (H) v_3	Probability (P) that $v_1 = v_3$
11/19	0.53	0.52 ± 0.05	0.26	0.50 ± 0.03	>0.7
10/12	0.67	.94 ± .15	.34	0.85 ± .07	> .6
11/19	1.0	.95 ± .09		0.62 ± .11	> .05
11/24	1.0	1.17 ± .12	.72	1.19 ± .17	> .8
9/30	1.22	1.19 ± .04	.25 ± 0.01	1.10 ± .09	> .3
10/9	1.0	1.20 ± .15	.42 ± .06	1.11 ± .09	> .6
11/13	4.0	3.6 ± .27	1.0	3.0 ± .16	> .2
11/16	4.0	4.3 ± .4	1.94	4.5 ± .3	> .7
9/23	5.0	5.1 ± 1.3	1.1	3.5 ± 1.4	> .3

[a] The slope is expressed as a positive function ±S.E. The equation for the curves is, therefore, $y = a - bx$, where b is the slope, y is the optical density, and x is expressed in 10^3 minutes.

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(3) N. B. Kurnick, THIS JOURNAL, **76**, 417 (1954).

(4) N. B. Kurnick, *Arch. Biochem.*, **29**, 41 (1950).

(5) N. B. Kurnick, *Arch. Biochem. and Biophys.*, **43**, 97 (1953).

TABLE II

VELOCITIES OF DEPOLYMERIZATION OF HEATED AND UNHEATED DNA BY RABBIT SERUM DNASE

Date and rabbit, (no.)	Ml. rabbit serum per 19 ml. substrate–serum mixture	Slopes[a] Unheated (P) v_1	Heated (H) v_2	Mixture (P) + (H) v_3	Probability (P) that $v_1 = v_3$
9/23 (1)	1/8	0.25 ± 0.03	0.11 ± 0.02	0.21 ± 0.02	>0.3
9/30 (1)	1/6	.35 ± .05	.17 ± .03	.33 ± .02	> .7
11/17 (2)	1/8	.375 ± .025	.11	.371 ± .030	> .9
11/12 (2)	1/5	.55 ± .07	.19 ± .04	.52 ± .05	> .7
11/13 (2)	1/6	.78 ± .10	0.25	.64 ± .03	> .3
10/12 (2)	1/4	1.06 ± .09	.30	.76 ± .09	> .02
11/13 (2)	1/2	1.62 ± .09	.34	1.10	< .001
8/25 (3)	1/4	2.10 ± .19	.70 ± .01	1.54 ± 0.06	> .01
11/18 (4)	1	2.65 ± .21	.6	2.20 ± .04	> .05
11/24 (5)	3/4	4.42 ± .42	1.55	3.29 ± .27	> .1
11/16 (6)	1	5.06 ± .50	1.4	3.77 ± .21	> .05
11/19 (7)	3	5.70 ± .28	1.3	5.02 ± .41	> .2

[a] See footnote to Table I.

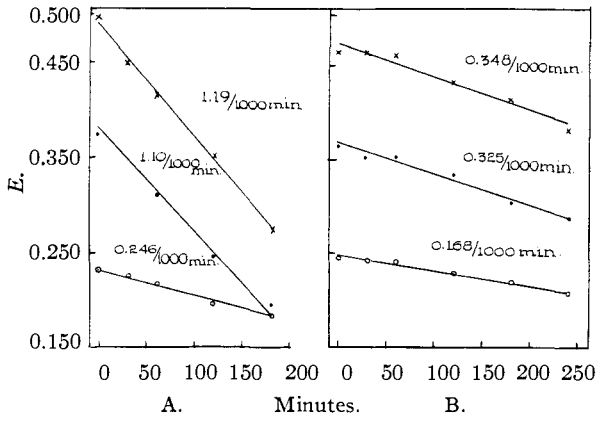

Fig. 1.—The slopes of the curves (given as a decimal along side each curve in the figure) indicate the rate of reduction in optical density at 640 mμ (= rate of depolymerization). In A, the enzyme is crystalline bovine pancreatic DNase. In B, the enzyme source is rabbit serum. In both A and B, the top curve (1) is for unheated substrate, the bottom curve (2) is for heated substrate, and the middle curve (3) is for the mixture of the heated and unheated substrates.

(Table I) and in only 3 of the 12 with rabbit serum (Table II) is the probability that the slopes of 1 and 3 are identical less than 0.05. It follows that in the mixture, the affinity of DNase for the unheated DNA is so much greater than for the heated that practically none of the DNase becomes bound up in the slower reaction with the heated substrate.

The ineffectiveness of heated DNA in competing for DNase with unheated DNA supports our previous suggestion[3] that heat causes intramolecular rearrangement (probably uncoiling) in the DNA molecule in addition to splitting it into smaller units, unlike the early effects of DNase depolymerization. Such steric alteration is more likely to account for the diminished affinity for DNase than is destruction of binding sites by heating.

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A Resin for the Selective Retention of Sulfhydryl Compounds

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A resin has been prepared by mercuration of a phenol–formaldehyde polymer that will selectively remove mercaptans from aqueous solutions. The mercaptans can be recovered by elution with dilute 2-mercaptoethanol or hydrogen sulfide solutions. Experiments 1 and 3 in Table I show that 13 and 20 mg., respectively, of glutathione (GSH) and cysteine are quantitatively retained by the resin and are recovered by elution with 2-mercaptoethanol. Oxidized glutathione (GSSG) is not retained by the resin at *p*H 7.0 (experiment 5) though it is partially retained at *p*H 3.0–3.5 (experiment 4). The retention of GSSG under the latter conditions is probably made possible by a reducing action of the resin which causes some reduction of GSSG to GSH. This view is supported by the observation that water extracts of the resin cause a reduction of equivalent amounts of iodine and GSSG. Experiment 6 shows that coenzyme A (CoA) is also retained by the resin and may be recovered by elution with solutions of potassium sulfide (0.1 *M*, *p*H 7.7). CoA is not eluted from the resin with 2-mercaptoethanol or quantitatively by acid solutions

TABLE I

Expt.	Compound	Milligrams put on resin	Milligrams recovered in water effluent	Milligrams recovered in 2-mercaptoethanol eluate
1	GSH	13.5	0[a]	12.0[a]
				12.8[b]
2	GSH (*p*H 7)	11.0	0.2[a]	...[c]
3	Cysteine·HCl	22.8	0	21.7
4	GSSG	31.1	21.7[a]	7.6[a]
			0.0[b]	7.4[b]
5	GSSG (*p*H 7)	11.7	11.7[a]	...[c]
6	CoA	21.5	0.76	21.2[d]
7	DL-Lysine	6.7	5.8	...[e]
8	DL-Serine	8.2	8.0	...[e]
9	L-Alanine	9.8	8.1	...[e]

[a] Determined by ninhydrin method. [b] Determined by alloxan method. [c] No elution with 2-mercaptoethanol was performed [d] Eluted with 0.1 *M* potassium sulfide (*p*H 7.7).