Cel C[4] and developed with 300 ml. of benzene–ethanol (50:1 by vol.). The extruded column was streaked with indicator (1% potassium permanganate in 10% sodium hydroxide[5]). The third zone (30 mm. from the column top) was sectioned, eluted with acetone and after concentration to a sirup, the product was separated from hot ethanol; yield 65 mg. of amorphous material, m.p. 119–123°, $[\alpha]^{25}D$ −21.5° (*c* 0.7, water); absorption spectra data[10]: λ_{max}^{EtOH} 268 mμ; λ_{max}^{KBr} 2.88, 2.97 μ (NH, OH), 5.75 μ (ester carbonyl), 6.10, 6.20, 6.86 μ (NH and purine ring), 8.15 μ (C–O–C of acetates).

Anal. Calcd. for $C_{33}H_{41}N_5O_{15}$: C, 49.81; H, 5.20; N, 8.80. Found: C, 49.36; H, 5.37; N, 8.52.

9-β-Cellobiosyladenine (VII).—Sirupy 6-acetamido-9-(hepta-*O*-acetyl-β-cellobiosyl)-purine (V, 10.6 g.) was dissolved at 0° in 150 ml. of methanol nearly saturated with ammonia at 0°. The solution was allowed to stand at this temperature for 21 hr. and was then concentrated under reduced pressure to a sirup, which was dissolved in 100 ml. of water and extracted thrice with 20-ml. portions of chloroform. The aqueous solution was evaporated under reduced pressure to a glassy solid; yield 3.6 g. (53%). This crude material was dissolved in 30 ml. of water and treated with 30 ml. of 10% picric acid. The solid yellow picrate that formed was separated by filtration, dissolved in water and the solution was stirred with Dowex-1 (carbonate form) until colorless. The filtered solution was concentrated to a thin sirup which was extracted with hot 1-butanol. After standing in an open beaker for 3 days at room temperature, prismatic crystals formed. The material was recrystallized from hot 1-butanol; yield 690 mg. (10.3%), dec. 304–307°, $[\alpha]^{26}D$ −11.4° (*c* 0.5, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 260 mμ; λ_{max}^{KBr} 2.87, 3.02 μ (OH, NH), 5.98 (H_2N—C=N) 6.18, 6.32, 6.75 μ (NH and purine ring), 9.30, 9.58, 9.75, 10.08 μ (C–O–C, C–OH); X-ray powder diffraction data[11]: 13.15vw, 10.53w, 8.31vw, 7.56w, 7.06m, 6.81w, 5.91vw, 5.55vw, 4.98w, 4.76m, 4.47s(3), 4.28m, 4.13s(2), 3.97m, 3.81w, 3.73w, 3.61m, 3.44vs(1), 3.26w, 3.18w, 3.00w.

Anal. Calcd. for $C_{17}H_{25}N_5O_{10}$: C, 44.44; H, 5.48; N, 15.25. Found: C, 44.42; H, 5.53; N, 15.57.

9-β-Cellobiosyladenine was also obtained from its acetate by deacetylation with *n*-butylamine according to the method of Reist and Baker[9]; yield 32%.

2,6-Diamino-9-β-cellobiosylpurine (VIII).—A suspension of 2,6-diacetamido-9-chloromercuripurine[6] (IV, 6 g.), 12 g. of cadmium carbonate and 8 g. of Celite[4] in 400 ml. of xylene was dried by azeotropic distillation of 100 ml. of the solvent. Hepta-*O*-acetyl-α-cellobiosyl bromide (9 g.) was added and the mixture was refluxed for 5.5 hr. The suspension was filtered, the filtrate was concentrated under reduced pressure and the residue and the filter cake were extracted with 250 ml. of hot chloroform. The chloroform extract was washed successively with 30% potassium iodide, water, dried over sodium sulfate and concentrated to a sirup; yield 7.4 g. This material was dissolved in 120 ml. of 0.1 *N* sodium methoxide in methanol and refluxed for 2 hr. The mixture was cooled, neutralized with acetic acid and concentrated to dryness. The residue was dissolved in 150 ml. of water and extracted thrice with 100-ml. portions of chloroform. The aqueous solution was treated with 25 ml. of 10% methanolic picric acid and allowed to stand overnight at 10°. The yellow crystalline precipitate was separated by filtration, washed with a little cold water and redissolved in 400 ml. of warm water. The solution was stirred with Dowex-1 (carbonate form) until colorless. The aqueous solution was filtered and concentrated under reduced pressure to a sirup. The sirup was extracted with 1-butanol. Crystals deposited from the solution upon standing at room temperature in an open beaker for 7 days and were recrystallized from methanol; yield 830 mg. (13.6%), m.p. 232–235°, $[\alpha]^{25}D$ −22° (*c* 0.4, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 254, 276 mμ; λ_{max}^{KBr} 2.95, 3.05 μ (NH, OH) 6.10, 6.25, 6.82 μ (NH and purine ring), 8.95, 9.25, 9.40, 9.70μ (C–O–C, C–OH); X-ray powder diffraction data[11]: 14.74w, 7.38vw, 7.02s(3), 6.37w, 5.56vw, 4.91w, 4.74s(2), 4.38m, 4.22s, 3.95w, 3.81vs(1), 3.30vw, 3.24m, 3.15w.

Anal. Calcd. for $C_{17}H_{26}N_6O_{10}$: C, 43.03; H, 5.53; N, 17.72. Found: C, 43.11; H, 5.52; N, 17.54.

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[CONTRIBUTION FROM THE BIOCHEMICAL RESEARCH LABORATORY, THE DOW CHEMICAL CO.]

Optical Rotatory Dispersion Studies on Polysaccharides. II. Conformation of Partially Methylated Cellulose in Solution[1]

BY W. BROCK NEELY

RECEIVED JANUARY 30, 1960

Optical rotatory dispersion techniques have been used to examine solutions of methylcellulose. The complex dispersion exhibited by certain solutions demonstrated that aggregation of the polymer was taking place. This association of the polysaccharide was enhanced at elevated temperatures and prevented at temperatures below 10°. The formation of these aggregates was also shown to be concentration dependent.

The use of optical rotatory dispersion techniques for investigating the solution properties of polysaccharides is a rather recent innovation. We wish to follow up our preliminary studies on methylcellulose[2] in this paper. In the past few years there has been an intensive investigation of the rotatory dispersion curves of proteins and polypeptides.[3] These studies were an attempt to correlate dispersion curves with the helix–coil transitions occurring in protein denaturation.

(1) Presented in part at the Symposium on Solution Properties of Cellulose and Cellulose Derivatives, Am. Chem. Soc. Meeting, Cleveland, Ohio, April, 1960.

(2) W. B. Neely, *Nature*, **185**, 159 (1960).

(3) See for example (a) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 498 (1956); (b) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957); (c) B. Jirgensons, *Arch. Biochem. and Biophys.*, **74**, 57 (1958); **74**, 70 (1958); **78**, 227 (1958); **78**, 235 (1958); (d) E. R. Blout, "Optical Rotatory Dispersion: Applications to Organic Chemistry," C. Djerassi, ed., McGraw-Hill Book Co., Soc., New York, N. Y., 1960, Chapter 17.

Rotatory dispersion data are usually fitted by a modification of the single term Drude equation (1). Such modifications are used for the purpose

$$[\alpha] = \frac{k}{\lambda^2 - \lambda_c^2} \qquad (1)$$

k and λ_c are constants

λ = wave length at which measurement is made

$[\alpha]$ = specific rotation at given wave length

of obtaining linear plots of α against λ resulting in methods for evaluating the constants λ_c and k from slope–intercept relations. Yang and Doty[3b] have shown that to obtain λ_c with great precision, it is more advantageous to use the modification of the Drude equation shown in (2). This

$$\lambda^2[\alpha] = \lambda_c^2[\alpha] + k \qquad (2)$$

form of representing dispersion data will be used in the present investigation. The value of λ_c has

been shown to correlate with the degree of organization in the protein molecule.[3] For denatured protein λ_c decreases in value when compared to the value for the native protein. Recently, there has been considerable doubt thrown on such a simple interpretation for the observed changes in λ_c.[3c,4] The present investigation, consequently, will not emphasize the numerical values of these dispersion constants. Instead, emphasis will be placed on the observed deviations of certain solutions from eq. 2. The interpretation of such results were based on the analogous studies using polypeptides.[3a,b] Yang and Doty[3b] demonstrated that the greater the deviation (complex dispersion) from linearity (simple dispersion) the greater the concentration of organized structure in the peptide.

It was felt that some of the properties of partially methylated cellulose in solution might be explained by the formation of aggregates. In a similar vein, it was felt that rotatory dispersion techniques offered the best method of exploring this possibility.

Experimental

Rotatory Dispersion.—The present studies were conducted with a Keston photoelectric polarimeter attached to the Beckman DU spectrophotometer. The usable range of wave lengths was from 400 to 650 mμ. For the temperature-controlled experiments, water from a temperature-controlled bath was circulated around a jacketed polarimeter tube.

Materials.—The material used for this study was Methocel MC[5] supplied by The Dow Chemical Co. This material is a partially methylated cellulose which has the characteristics of being more soluble in cold water than in hot water. The polymer is obtainable in various viscosity grades; the present investigation utilized Methocel MC with an intrinsic viscosity of 1.6 (corresponding roughly to a molecular weight of 35,000) and a methoxyl analysis of 29.5% (1.8 OCH_3 groups/anhydroglucose unit). Two methods of solubilizing the polysaccharide were used. "Solubilized at room temperature," will refer to Methocel MC dispersed in boiling water and cooled to room temperature. "Solubilized at 0°," will refer to Methocel MC dispersed in boiling water and cooled in the refrigerator (0–5°) overnight and then maintaining at room temperature.

Analytical.—The methylcellulose was analyzed by the anthrone colorimetric procedure developed by Samsel and DeLap.[6]

Results and Discussion

The rotatory dispersion curves of the methylcellulose solutions solubilized at 0° and at room temperature have previously been described.[2] Plotting the dispersion data by eq. 2 gives the curves shown in Fig. 1. Curve D through B represent the dynamic nature of the polymer when it is solubilized at room temperature. Curve D is the initial complex dispersion which slowly changes on standing to curve C (1 hour) and finally to the linear relationship B (16 hours). On shaking the solution represented by B, the original curve D is returned. Curve A represents Methocel MC solubilized at 0°. Both A and B follow the simple one-term Drude equation given by materials which are randomly dispersed in solution.

The anomalous dispersion represented by curve D, Fig. 1, can be explained on the basis of molecular association causing aggregates which slowly settle out of solution leaving the randomly dispersed material behind. This would also provide an explanation why the dispersion curve B, Fig. 1, never attains curve A, Fig. 1. Due to association, part of the Methocel MC is removed leaving a solution whose concentration is decreased, thus giving reduced values for the observed rotation.

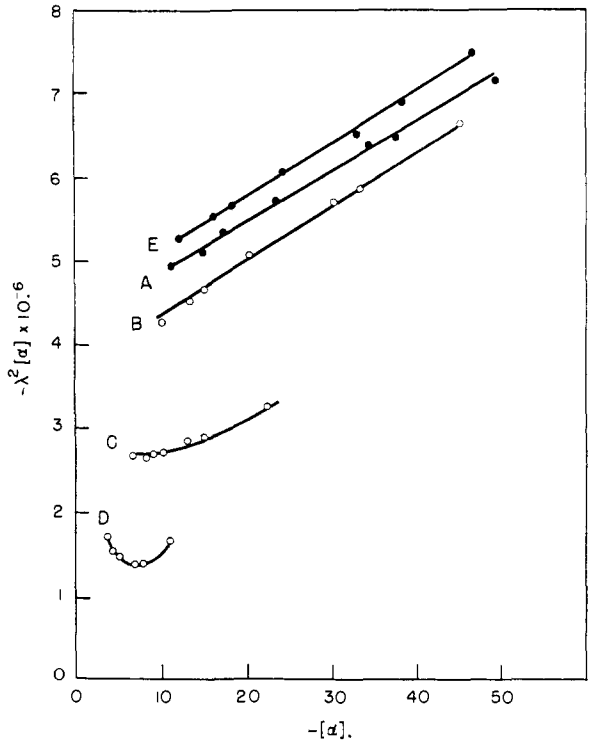

Fig. 1.—Modified Drude plots of rotatory dispersion data on various methylated cellulose solutions. See text for discussion of the curves.

This point was checked by subjecting both types of Methocel MC to centrifugation at 8,000 g. for 0.5 hour and then determining the dispersion curves on the supernatant. The specific rotations were based on concentrations of Methocel MC in the supernatant measured by the anthrone analytical procedure.[6] The Drude plots are shown in Fig. 1, curve E. Both solutions, solubilized at room temperature and solubilized at 0°, give identical results when corrected for loss by aggregate formation. Both solutions also represent random dispersion of methylcellulose molecules. The loss in weight due to aggregate formation in either case was small and as expected the loss in weight from the room temperature solution was the greater.

These particular experiments were most gratifying as they provided a base line which was reproducible and could be used as a reference line for future comparison.

The effect of temperature on the dispersion curves and hence on the state of aggregation of the Methocel MC was checked. These results are summarized in Fig. 2. As the temperature was lowered to 10° the dispersion approached the base line. The temperature bath was unable to maintain a lower temperature; however, it is to be expected that at 0° the curve would coincide with the base line indicating that the methylcellulose was completely dispersed with no aggregates being

(4) B. Jergensons and T. Ikenaka, *Makromol. Chem.*, **31**, 112 (1959).

(5) Registered Trademark of The Dow Chemical Co., Midland, Mich.

(6) E. P. Samsel and R. H. DeLap, *Anal. Chem.*, **23**, 1795 (1951).

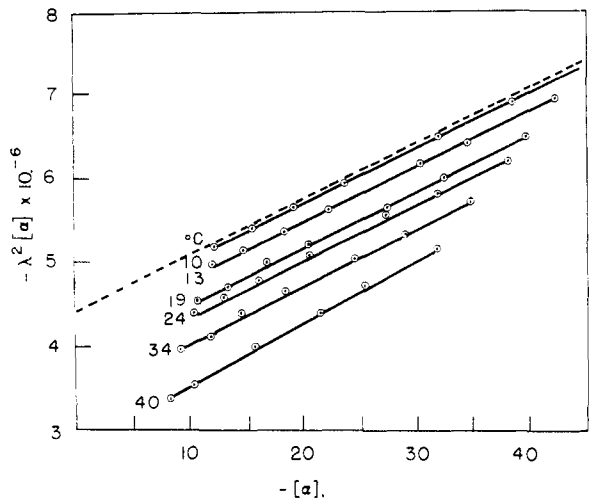

Fig. 2.—Modified Drude plots of methylated cellulose solutions at various temperatures. The dashed line represents the base plot obtained on the supernatant from a centrifuged 2% solution.

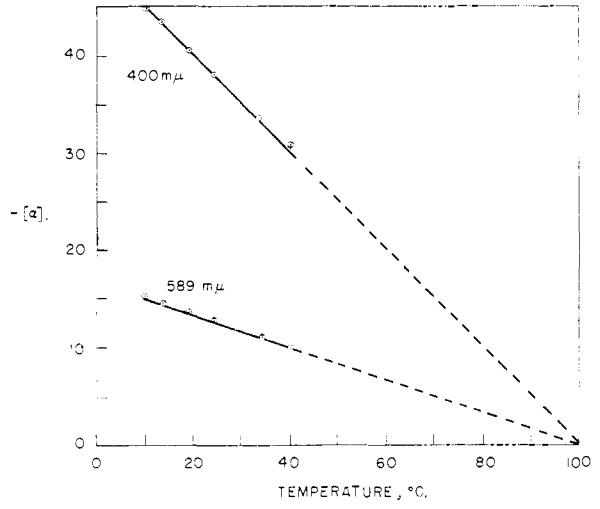

Fig. 3.—Plot of $[\alpha]$ at 400 mμ and 589 mμ against temperature of a solution of Methocel MC.

present. As the temperature was raised the dispersion curve progressed to lower values parallel with the base line. This would indicate that as the temperature increased the amount of aggregation increased. The aggregates as they were formed settle out leaving the randomly dispersed Methocel MC. Above 45° the solution became very cloudy and the readings lost their significance. This effect of temperature was completely reversible. One more interesting observation could be made from these results. Plotting the values of specific rotation against temperature gave a linear relationship, Fig. 3. This line on extrapolation crossed the temperature axis at 100° indicating that at this temperature there would be zero rotation. Methylcellulose was insoluble at 100° and obviously would not show any optical activity.

The dispersion of a 5% solution of Methocel MC solubilized at 0° was studied at 10°. These results are shown in Fig. 4. This solution exhibited considerable anomalous dispersion even at 10°. The

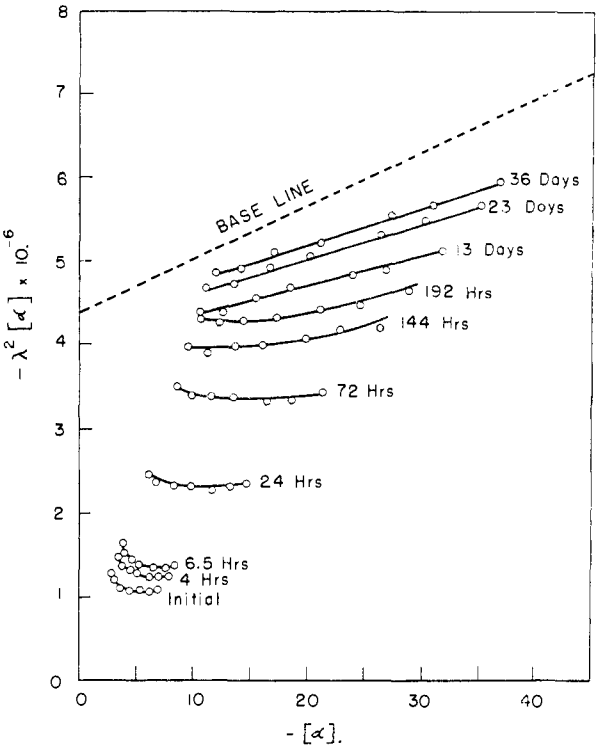

Fig. 4.—Modified Drude plots of a 5% solution of methylated cellulose at 10°. This demonstrated the slow change from a complex dispersion to a linear type dispersion with time.

dispersion gradually changed and approached the base line as the aggregates slowly became completely hydrated and assumed the characteristics of the randomly dispersed solutions discussed previously for the 2% solution of Methocel MC at 10°.

Aggregate formation appeared to be dependent on concentration. This conclusion was supported by the observations on the dilution of the above 5% 0° solubilized solution to 2.5%. The diluted solution at 20° required only 2 hours to approach the equilibrium base line. The base line in this case was the dispersion curve of the 2% solution of Methocel MC at 19° (see Fig. 2).

The 2% solution of Methocel solubilized at room temperature was examined in a more critical fashion. These results again illustrated the anomalous dispersion and the gradual change to the linear curve. The equilibrium dispersion at 20° of this particular solution (curve B, Fig. 1) lies slightly below the curve for Methocel MC solubilized at 0° (curve A, Fig. 1) at this temperature. This illustrated the point that more aggregates were present in Methocel MC solubilized at room temperature than material which had been solubilized at 0°. The concentration dependence of this phenomenon was again demonstrated by the fact that when a 2% solution of Methocel MC solubilized at room temperature was diluted to 1% the dispersion rapidly (2.5 hours) approached the equilibrium point represented by curve A, Fig. 1.

That aggregation of the Methocel MC molecule was a dynamic process was further illustrated from the results of the following experiments: 1. A 2% solution of Methocel MC solubilized at room tem-

perature was centrifuged at 8000 g. for 0.5 hour. The rotations on the supernatant were observed. This solution was maintained at room temperature for a period of 9 days and the dispersion curve was repeated. This was again centrifuged and a dispersion curve made. The results are summarized in Table I.

TABLE I

RESULTS OF TIME DEPENDENCE IN FORMATION OF AGGREGATES AT ROOM TEMPERATURE

	Solution	$[\alpha]_{382.6}$[a]	Dispersion
1	2% Solubilized at room temp. and centrifuged	46.2°	Linear
2	Soln. 1 standing 9 days	33.6	Complex
3	Soln. 2 centrifuged	44	Linear

[a] Not corrected for loss of Methocel MC due to aggregation.

2. A 2% solution of Methocel MC solubilized at 0° in a period of two weeks slowly became aggregated. This was evidenced by the slow drift in the dispersion curve of the aged solution back to the original base line. It was not as aggregated as the room temperature material, but it did indicate that given a sufficient length of time the two solutions would undoubtedly have identical equilibrium states.

Conclusions

In order to obtain a true randomly dispersed solution of partially methylated cellulose, the temperature must be lowered to at least 10° and preferably colder. The results with the 5% solution of methylcellulose also indicated that as the concentration of the polymer increases, a greater length of time is required for complete hydration. At this low temperature, the molecule becomes enveloped with a bound water layer which prevents any aggregation. As the temperature is raised, part of the water envelope is disrupted. Such a situation provides an opportunity for the Methocel MC molecules to become associated in a sheet-like structure. This aggregate slowly settles to the bottom of the solution. As the temperature continues to increase the polysaccharide becomes completely dehydrated and falls out of solution.

Yang and Doty[3b] were able to show a similar type association for low molecular weight polypeptides. They also demonstrated[3b] that the formation of these intermolecular bonded structures was concentration dependent. A similar situation was found to be true for solutions of methylcellulose. As the concentration becomes greater, more opportunity for aggregation is provided and the reverse situation occurs as the concentration is decreased.

MIDLAND, MICH.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y.]

Preparation of Polymeric Condensation Products Containing Functional Thiol Side Chains. Polyurethans[1]

BY C. G. OVERBERGER AND HERBERT ASCHKENASY[2]

RECEIVED FEBRUARY 5, 1960

The purpose of this study was to develop a general method of synthesis for a polyurethan and a polyester containing free sulfhydryl groups. Addition of benzyl mercaptan to dimethylitaconate followed by reduction to the glycol II gave a useful monomer. A polyester was prepared from this glycol and adipoyl chloride, but the benzyl group could not be removed without degradation of the polymer. A series of polyurethans was prepared and characterized by reaction of the glycol II with available diisocyanates. The benzyl group was successfully removed from the polyurethan made with the glycol and tolylene 2,4-diisocyanate with sodium in a mixed solvent of liquid ammonia and *n*-propylamine.

Polymers containing sulfhydryl groups are compounds of considerable interest for a variety of reasons. The effect of environment on the oxidation-reduction potentials of the sulfhydryl–disulfide system is of biochemical significance.[3] Thiols combine with heavy metals to form mercaptides, a property which has been used for the detection of thiol groups in biological systems as well as for the preparation of exchange resins for the removal of heavy metal ions.[4]

The sulfhydryl group is essential to the activity of a number of enzymes in biological processes.[5] Oxidation of the sulfhydryl group in many cases inactivates the enzyme. Synthetic polymers containing thiol groups might be of considerable usefulness as reducing agents in the thiol-disulfide system as Overberger and Lebovits[6] have demonstrated by reactivating urease, inactivated by chemical oxidation, with a sulfhydryl-containing vinyl polymer.

In addition, it has been established empirically that compounds having a sulfhydryl group are the most efficient protectors of living organisms against ionizing radiation,[7] and a great variety of compounds, preponderantly monomeric in nature, have been prepared for testing in this connection. A

(1) This is the 19th in a series of papers on new monomers and polymers; for this previous paper in this series, see C. G. Overberger and J. E. Mulvaney, THIS JOURNAL, **81**, 4697 (1959).

(2) This paper comprises part of the thesis presented by Herbert Aschkenasy in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the graduate school of the Polytechnic Institute of Brooklyn.

(3) E. S. G. Barron, Vol. XI, "Advances in Enzymology," Interscience Publishers Inc., N. Y., 1951, pp. 219 et seq.

(4) J. R. Parrish, *Chemistry & Industry*, 137 (1956); H. P. Gregor, D. Dolan and G. K. Hoeschele, THIS JOURNAL, **77**, 3675 (1955).

(5) L. Hellerman, M. C. Perkins and W. M. Clark, *Proc. Natl. Acad. Sci. U. S.*, **19**, 855 (1933); E. S. G. Barron and T. B. Singer, *Science*, **97**, 358 (1943).

(6) C. G. Overberger and A. Lebovits, THIS JOURNAL, **78**, 4792 (1956).

(7) Proposed Anti-Radiation Drug Program, Part II, Dept. of Nuclear Medicine, Walter Reed Army Institute of Research, Walter Reed Medical Center, Washington, D. C.