

Viscosities of Acacia and Sodium Alginate after Sterilization by Cobalt-60

ALAN W. HARTMAN, RUSSELL U. NESBITT, Jr., F. MAURIECE SMITH, and NOEL O. NUESSELE*

Abstract □ Acacia and sodium alginate powders were sterilized by cobalt-60 irradiation to a total dose of 2.5 Mrad with no increase in residual radioactivity of the gums as a result of the treatment. Viscosity measurements showed an 11% reduction in the viscosity of acacia in water and a 16% decrease in the intrinsic viscosity in barium chloride solution due to the irradiation. Viscosities of sodium alginate solutions in 0.1 N sodium chloride showed a 70% decrease in intrinsic viscosity, indicating changes in the molecular structure corresponding to degradation to 30% of the original degree of polymerization. This dose of γ -radiation from cobalt-60 is not an appropriate method of sterilization for these gums.

Keyphrases □ Acacia—viscosity changes after sterilization by cobalt-60 □ Sodium alginate—viscosity changes after sterilization by cobalt-60 □ Irradiation, cobalt-60—effects on viscosity of acacia and sodium alginate □ Sterilization, cobalt-60—effects on viscosity of acacia and sodium alginate

Acacia, a dried exudate from species of *Acacia*, obtained by tapping the trees, is frequently sun bleached and sorted on the ground. Acacia consists primarily of a mixture of arabic acid salts which, on hydrolysis, yields L-arabinose, L-rhamnose, D-galactose, and glucuronic acid. Sodium alginate is extracted from brown seaweed by aqueous alkali and consists of a polymer of D-mannuronic and L-guluronic acids. Both of these vegetable gums, natural plant hydrocolloids, are subject to microbial attack and, because of their nature and method of production, contain microbes capable of causing spoilage of products incorporating them.

DISCUSSION

The primary use of these gums is to increase viscosity of aqueous systems. Solutions of gums, as with other high polymers, exhibit a great increase in viscosity over that of the solvent alone even when the concentration of the gum is small. It was suggested (1, 2) that viscosity could be quantitatively correlated to the molecular weight (M) of the polymer according to:

$$[\eta] = KM \quad (\text{Eq. 1})$$

where $[\eta]$ is the intrinsic viscosity, and K is a constant. This equation was later modified (3, 4) to the following expression:

$$[\eta] = K'M^a \quad (\text{Eq. 2})$$

where K' and a are also constants. Since these equations are based on empirical findings, this is not an absolute method for obtaining molecular weights. The method must be established for each particular polymer-solvent system at a definite temperature by reference to some other absolute method of molecular weight determination. Furthermore, the method is only applicable to linear polymers. Capillary viscometers are generally used, and the results are expressed as relative viscosity, η_r :

$$\eta_r = \eta/\eta_0 \quad (\text{Eq. 3})$$

where η is the viscosity of the polymer solution, and η_0 is the vis-

Table I—Viscosity of Aqueous Acacia Solutions at 30°^a

Concentration, g/100 ml	Nonirradiated, cps	Irradiated, cps	Change, %
2	1.707 ± 0.021	1.546 ± 0.015	-9.43
5	2.749 ± 0.033	2.448 ± 0.018	-10.95
10	4.955 ± 0.076	4.408 ± 0.033	-11.04

^a Mean and relative standard derivation of five determinations.

cosity of the solvent. The older nomenclature is used here since the present work is based on the older literature. However, in determining the molecular weight, several other viscosities are used:

$$\text{specific viscosity} = \eta_{sp} = (\eta - \eta_0)/\eta_0 \quad (\text{Eq. 4})$$

$$\text{reduced viscosity} = \eta_{red} = \eta_{sp}/c \quad (\text{Eq. 5})$$

$$\text{intrinsic viscosity} = [\eta] = (\eta_{sp}/c)_{c \rightarrow 0} \quad (\text{Eq. 6})$$

and

$$[\eta] = [(\ln \eta_r)/c]_{c \rightarrow 0} \quad (\text{Eq. 7})$$

where c is equal to grams of polymer/100 ml of solution. In effect, molecular weights are related to the reduced viscosity at infinite dilution. At finite concentrations of polymer, the reduced viscosity is increasingly dependent on concentration as the concentration increases. To obtain the intrinsic viscosity, one extrapolates to zero concentration both a plot of η_{sp}/c versus concentration and a plot of $(\ln \eta_r)/c$ versus concentration. The midpoint of the two intercepts is the intrinsic viscosity.

To obtain the constants in Eq. 2, the log of $[\eta]$ is plotted versus the log of the molecular weight of various fractions of the polymer (5). The slope of the linear relationship is equal to a and the intercept is K' . If the following expression is valid for a monodisperse polymer:

$$[\eta]_m = KM^m \quad (\text{Eq. 8})$$

where m indicates monodispersity, for a polydisperse polymer the relationship becomes:

$$[\eta]_p = K \left[\frac{\sum N_j M_j^{Ha}}{\sum N_j M_j} \right] = K \bar{M}_v^a \quad (\text{Eq. 9})$$

where p indicates polydispersity, and K and a have the same values as in Eq. 8 (6). The viscosity-average molecular weight is indicated by \bar{M}_v . For randomly coiled polymers, a varies from 0.5 in a θ solvent to a maximum of about 1, generally being between 0.6 and 0.8. Typical values of K' range from 0.5 to 5×10^{-4} . For many polymers, \bar{M}_v is 10-20% lower than the weight-average molecular weight; but if $a = 1$, the viscosity-average molecular weight is the same as the weight-average molecular weight (7).

Polyelectrolyte polymers such as acacia and sodium alginate exhibit expanded conformation due to electrostatic repulsion of the charged sites as they lose mobile ions to the solution, and this expansion increases as the dilution increases. The expansion changes the frictional characteristics of the polymer; thus, while the reduced viscosity of a nonionic polymer gradually increases with increasing concentration, the reduced viscosity of a polyelectrolyte polymer markedly increases with decreasing concentration. The addition of simple salts to the polyelectrolyte solution creates a Donnan equilibrium, suppressing the loss of mobile ions from the polymer, preventing expansion, and enabling it to assume the random coiled configuration required for the use of Eq. 1.

In a previous study (8), it was shown that 2.5 Mrad of cobalt-60

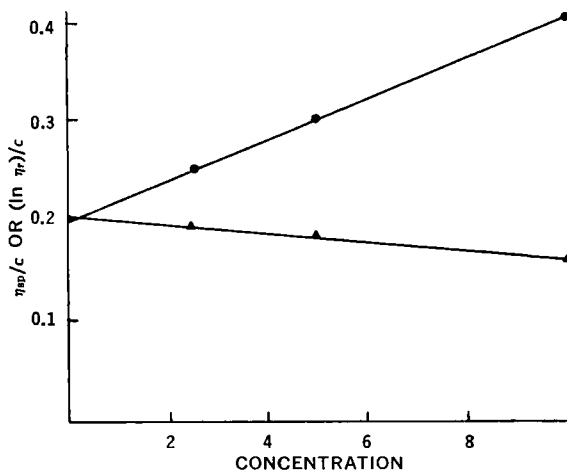


Figure 1—Viscosity data for nonirradiated acacia. Key: ●, η_{sp}/c ; and ▲, $(\ln \eta_r)/c$.

radiation was sufficient to sterilize selected colorants without causing a discernible change in their visible, UV, and IR absorption spectra. In the present study, acacia and sodium alginate powders were subjected to 2.5 Mrad of γ -irradiation from cobalt-60 and solutions of the treated gums were studied for effects on viscosity. A recently published report showed that sodium alginate powder subjected to 2.5 and 5 Mrad of cobalt-60 radiation gave solutions of lowered viscosity (9).

EXPERIMENTAL

The following materials were used: acacia USP, powder¹; sodium alginate powder¹; polysorbate 80 USP²; trypticase soy agar³; polyethylene vials, 4 dram with snap cap⁴; and ointment jars, 120 ml, opal glass with screw caps⁵.

Irradiation—Gums were placed in polyethylene vials which had been ultrasonically cleaned. The vials were completely filled to eliminate excessive head-space and were irradiated⁶ as previously described for approximately 10 hr to a total dose of 2.5 Mrad. Larger quantities were irradiated in completely filled jars. The cobalt-60 source consisted of 10 stainless steel clad cylinders of cobalt metal, each measuring 29.3 cm in length and 1.1 cm in diameter, arranged in a cylindrical array having a diameter of 14.0 cm.

The dose rate of the array was established by the National Bureau of Standards from a Fricke dosimeter placed in the center of the array, and current dose rates were calculated from this value using a half-life of 5.26 years for cobalt-60. The dose rate as of June 1973 was 2.53×10^5 rad/hr for samples, as in this study, placed in the same position as the dosimeter. Exposure time for a 2.5-Mrad dose in June 1973 was 9 hr and 53 min, and the length of irradiation for subsequent samples was adjusted according to the date of exposure.

Sterility Testing—A sample of gum was aseptically obtained and suspended in 10 ml of sterile saline solution (0.9% w/v) containing 1% (w/v) polysorbate 80. An aliquot of this suspension was transferred to a trypticase soy agar plate. Samples of nonirradiated sodium alginate were 0.2 g, and those of irradiated material were 0.3 g. Aliquots placed on the plates were 2.5 ml. The acacia samples were 1 g and the aliquots were 3 ml. Three irradiated and three nonirradiated samples of each gum were tested. Plates were incubated at 30° and checked daily for up to 14 days.

Induced Radiation—Radiation measurements⁷ were made on

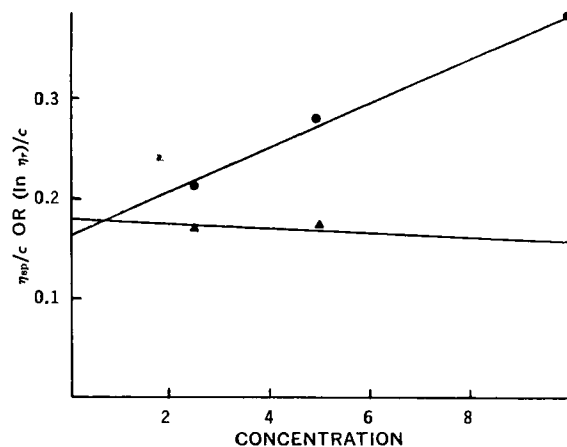


Figure 2—Viscosity data for cobalt-60 irradiated acacia. Key: ●, η_{sp}/c ; and ▲, $(\ln \eta_r)/c$.

1-g samples of the gums, irradiated and nonirradiated. The counting period was 15 min, and each sample was counted three times.

Viscosity—Solutions of acacia, irradiated and nonirradiated, were prepared containing 2, 5, and 10 g/100 ml of solution in water and containing 2.5, 5, and 10 g/100 ml in 10 mM barium chloride solution. Solutions of irradiated and nonirradiated sodium alginate in 0.1 N sodium chloride solution were prepared containing 0.1, 0.3, and 0.5 g/100 ml of solution. All solutions were preserved by the addition of 0.1% methylparaben, and the solutions were allowed to solvate for 24 hr before viscosity measurements⁸ were made, except for acacia in barium chloride solutions which were allowed to solvate for 2 weeks.

Viscosities of aqueous acacia solutions were determined at 30°, of acacia in barium chloride solutions at 42°, and of sodium alginate solutions at 25°; all temperatures were $\pm 0.02^\circ$. Barium chloride and sodium chloride were used to suppress the extension of the polyelectrolytes. Donnan and Rose (10) found that the use of 0.1 N sodium chloride swamped the electroviscous effect of sodium alginate, while barium chloride was utilized in a viscosity study of acacia (11).

RESULTS

Sterility Tests—All plates containing the nonirradiated gums, 50 mg of sodium alginate or 300 mg of acacia, showed copious

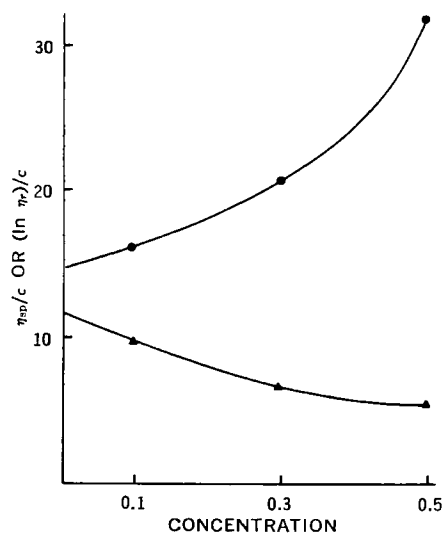


Figure 3—Viscosity data for nonirradiated sodium alginate. Key: ●, η_{sp}/c ; and ▲, $(\ln \eta_r)/c$.

¹ Ruger Chemical Co., Irvington, N.J.

² Tween 80, I. C. I. America, Inc., Wilmington, Del.

³ Baltimore Biological Laboratories, Baltimore, Md.

⁴ Polyvials, Olympia Plastics, Los Angeles, Calif.

⁵ Brockway Glass Co., Brockway, Pa.

⁶ The cobalt-60 irradiation was performed at the Research Reactor Facility, Columbia, Mo.

⁷ The measurements were made using a Nuclear Chicago model 1620 A scaler with an ultrathin window tube and a Nuclear Chicago model 1810 single-channel analyzer; Nuclear Chicago, Chicago, Ill.

⁸ Obtained using Cannon-Fenske viscometers, Fisher Scientific Co., Pittsburgh, Pa.

Table II—Viscosities of Acacia in 10 mM Barium Chloride Solution at 42°^a

Concentration, g/100 ml	η_r	η_{sp}	η_{sp}/c , g/100 ml	$(\ln \eta_r)/c$, g/100 ml
Nonirradiated				
2.5	1.63 ± 0.01	0.627 ± 0.013	0.250 ± 0.005	0.195 ± 0.003
5	2.52 ± 0.02	1.52 ± 0.02	0.303 ± 0.005	0.185 ± 0.002
10	5.07 ± 0.08	4.07 ± 0.08	0.407 ± 0.008	0.162 ± 0.002
Cobalt-60 Irradiated				
2.5	1.53 ± 0.01	0.528 ± 0.013	0.211 ± 0.005	0.169 ± 0.004
5	2.39 ± 0.02	1.39 ± 0.02	0.278 ± 0.004	0.174 ± 0.002
10	4.81 ± 0.03	3.81 ± 0.03	0.381 ± 0.003	0.157 ± 0.001

^a Mean and relative standard deviation of five determinations.

growth within 72 hr. Plates containing the irradiated material, 75 mg of sodium alginate or 300 mg of acacia, showed no growth under the test conditions and were regarded as sterile.

Induced Radiation—No radioactivity of acacia and sodium alginate was induced by the cobalt-60 sterilization process.

Viscosity—Viscosities of acacia solutions in water (Table I) showed a decrease of about 10% as a result of irradiation, and the solutions of irradiated material exhibited a somewhat darker color. The viscosities of acacia in 10 mM barium chloride solution (Table II) showed a slight decrease for the irradiated material. The relationship of η_{sp}/c and $(\ln \eta_r)/c$ to concentration appears to be linear for both the nonirradiated (Fig. 1) and irradiated (Fig. 2) acacia in barium chloride solution. The plot of η_{sp}/c versus concentration has a correlation coefficient of 0.996, a slope of 0.0208, and an intercept of 0.1986 for the nonirradiated acacia. Similar values for the irradiated acacia are 0.996, 0.0224, and 0.1592. The plot of $(\ln \eta_r)/c$ against c gives values of -0.9869, -0.0043, and 0.2057 for nonirradiated acacia and -0.794, -0.0019, and 0.1779 for the irradiated gum.

Three experimental points on each line are not sufficient for establishing the intrinsic viscosity, $[\eta]$, but do provide enough information to estimate an apparent intrinsic viscosity, which being dependent on molecular structure is a good criterion for judging the suitability of this method of sterilization. In this respect, an apparent intrinsic viscosity would be a better criterion than a viscosity based on expanded conformation of the polyelectrolyte. If the further assumption is made that the relationship of Eq. 1 exists between the apparent intrinsic viscosity and the molecular weight (despite finding no such relationship in a perusal of the literature), the effects of the sterilization treatment on the acacia molecule may be compared to the effects on the sodium alginate structure. Thus, the apparent $[\eta]$ obtained is 0.202 for nonirradiated acacia and 0.169 for irradiated acacia, indicating that the irradiation sterilization with 2.5 Mrad of cobalt-60 results in the apparent depolymerization of acacia to the extent of about 16%.

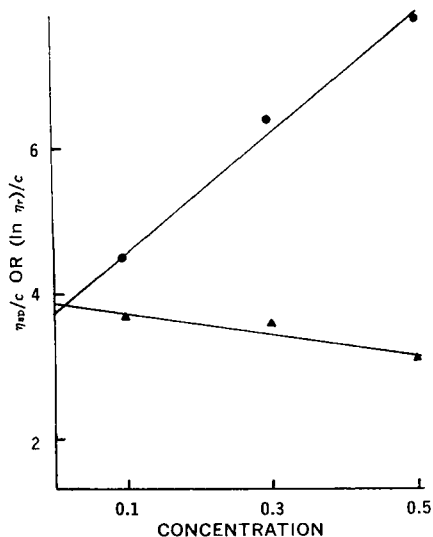


Figure 4—Viscosity data for cobalt-60 irradiated sodium alginate. Key: ●, η_{sp}/c ; and ▲, $(\ln \eta_r)/c$.

Viscosities of the sodium alginate solutions in 0.1 N sodium chloride solution are shown in Table III. The decrease in values of the solutions of irradiated gum is quite apparent. The relationship of η_{sp}/c and $(\ln \eta_r)/c$ to concentration is not linear for nonirradiated alginate (Fig. 3), which is in agreement with the investigation of Donnan and Rose (10). These curves were extrapolated to the intercept, and the midpoint of the intercepts was taken as the best value for the apparent intrinsic viscosity. The linearity of these same plots for the irradiated alginate (Fig. 4) is indicative of apparent depolymerization as a result of the cobalt-60 treatment. By using the nine values, the plot of η_{sp}/c versus c was found to have a correlation coefficient of 0.992, a slope of 8.22, and an intercept of 3.75. The plot of $(\ln \eta_r)/c$ yielded values of -0.935, -1.35, and 3.89, respectively. The apparent $[\eta]$ obtained for the irradiated alginate was 3.82, while that of the nonirradiated gum was 12.8. Irradiated sodium alginate and its solutions also exhibit a greenish color.

The relationship of molecular weight and intrinsic viscosity of sodium alginate solution, using the data of Donnan and Rose (10), is shown in Fig. 5. This relationship is linear and therefore follows Eq. 1. When using $K = 7.97 \times 10^{-5}$ (12), calculations indicate the \bar{M}_v of sodium alginate to be approximately 161,000 prior to the cobalt-60 irradiation and approximately 47,900 afterward. Donnan and Rose found the degree of polymerization (D.P.) to be related to the intrinsic viscosity by the following expression:

$$\frac{\text{D.P.}}{[\eta]} = 58 \quad (\text{Eq. 10})$$

By using Eq. 10 and an equivalent weight of 216, the D.P. and \bar{M}_v of the nonirradiated alginate are found to be 742 and 160,000, respectively; the values for the irradiated material are calculated to be 222 and 47,900, which correlate well. The cobalt-60 sterilization

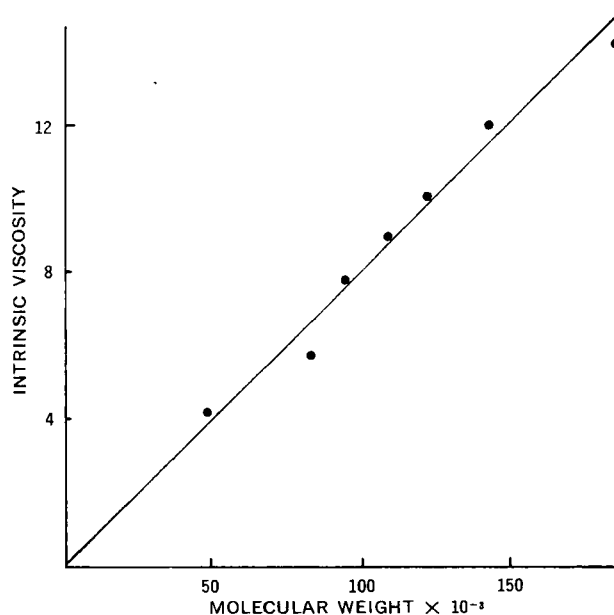


Figure 5—Molecular weight and intrinsic viscosity relationship from the data of Donnan and Rose (10).

Table III—Viscosities of Sodium Alginate in 0.1 N Sodium Chloride Solution at 25°^a

Concentration, g/100 ml	η_r	η_{sp}	η_{sp}/c , g/100 ml	$(\ln \eta_r)/c$, g/100 ml
Nonirradiated				
0.1	2.62 ± 0.01	1.62 ± 0.01	16.2 ± 0.11	9.62 ± 0.04
0.3	7.23 ± 0.08	6.23 ± 0.08	20.8 ± 0.27	6.59 ± 0.07
0.5	16.8 ± 0.11	15.8 ± 0.11	31.7 ± 0.21	5.65 ± 0.02
Cobalt-60 Irradiated				
0.1	1.45 ± 0.02	0.449 ± 0.016	4.49 ± 0.16	3.71 ± 0.11
0.3	2.92 ± 0.05	1.92 ± 0.05	6.39 ± 0.16	3.57 ± 0.03
0.5	4.89 ± 0.07	3.89 ± 0.07	7.78 ± 0.15	3.17 ± 0.03

^a Mean and relative standard deviation of three determinations.

treatment apparently has reduced the polymer to an average of 30% of its former degree of polymerization. The use of 216 for the equivalent weight takes into account the fact that sodium alginate exists in a hydrated form in which one molecule of water is associated with each mannuronide or guluronide unit.

The use of cobalt-60, 2.5 Mrad of γ -radiation applied over a 10-hr period, is not a suitable sterilization method for either of these vegetable gums. The apparent $[\eta]$ of acacia is reduced 16% and that of sodium alginate 70%. These viscosities represent a similar reduction in molecular weight; thus, the irradiated sodium alginate has the apparent $[\eta]$ of a fraction having a molecular weight 30% of the original molecule. Since the intent of this investigation was to determine the suitability of the use of cobalt-60 as a sterilization method, no study was made to ascertain what specific changes in the molecules actually occurred, such as depolymerization or changes in the carboxylic groups.

The reduction in apparent intrinsic viscosity for sodium alginate is over fourfold that occurring with acacia. Since each uronide unit in the alginate structure is accompanied by one molecule of water, it is possible that these water molecules actively participate in the greater depolymerization of sodium alginate. An alternative to depolymerization or extensive cleavage of the polymer is a change in the carboxylic groups present on the chain; these groups are more numerous in the alginate molecule, having an equivalent weight of 216, than in acacia, having an equivalent weight on the order of 1000–1200 (13). While the latter is not proposed as the mechanism for the greater reduction in viscosity of sodium alginate than of acacia as a result of the cobalt-60 sterilization process, it appears more plausible than chain cleavage by the water molecules.

REFERENCES

(1) H. Staudinger and W. Heuer, *Ber. Deut. Chem. Ges.*, **63**, 222(1930).

- (2) H. Staudinger and W. Nodzu, *ibid.*, **63**, 721(1930).
 (3) H. Mark, "Der Feste Körper," Leipzig, Germany, 1938.
 (4) R. Houwink, *J. Prakt. Chem.*, **157**, 15(1940).
 (5) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, New York, N.Y., 1953, pp. 308–314.
 (6) P. F. Onyon, in "Techniques of Polymer Characterization," Academic, New York, N.Y., 1959, chap. 6.
 (7) F. W. Billmeyer, Jr., "Textbook of Polymer Science," 2nd ed., Wiley-Interscience, New York, N.Y., 1971, pp. 84–90, 95, 96.
 (8) B. Chang, W. G. Haney, Jr., and N. O. Nuessele, *J. Pharm. Sci.*, **63**, 758(1974).
 (9) D. Coates and G. Richardson, *Can. J. Pharm. Sci.*, **9**, 60(1974).
 (10) F. G. Donnan and R. C. Rose, *Can. J. Res. Sect. B*, **28**, 105(1950).
 (11) H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloid-Beih.*, **36**, 429(1932).
 (12) M. Kurata and W. H. Stockmayer, *Fortschr. Hochpolym.-Forsch.*, **3**, 196(1963).
 (13) C. L. Mantell, in "Natural Plant Hydrocolloids," vol. 11, Advances in Chemistry Series, American Chemical Society, Washington, D.C., 1954, pp. 20–32.

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* To whom inquiries should be directed.