GI Pharmacology of Polyethyleneimine I: Effects on Gastric Emptying in Rats

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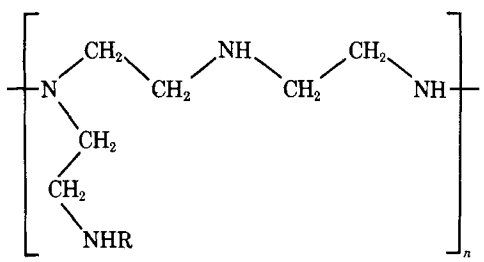
Abstract □ Experiments were conducted to determine the effect of ingested polyethyleneimine upon gastric emptying of the fasted rat. Emptying was evaluated by the phenolsulfonphthalein and resin bead methods. The two techniques gave comparable results; both showed that this agent inhibited gastric emptying. A delay in gastric emptying could be detected within 15 min of intubation. The effect was dose related, quite long lasting (~4 hr), and reversible. Commercially available, branched polyethyleneimines were highly active, but the linear polyethyleneimine was without observable effect. A branched polyethyleneimine derivative with all primary amine sites selectively acetylated also was inactive.

Keyphrases □ Polyethyleneimines, various—effect on gastric emptying, phenolsulfonphthalein and resin bead methods compared, rats □ Gastric emptying—effect of various polyethyleneimines, phenolsulfonphthalein and resin bead methods compared, rats □ Polyamines—various polyethyleneimines, effect on gastric emptying, phenolsulfonphthalein and resin bead methods compared, rats

Commercial polyethyleneimines are used as fungicides in shampoo (1), as bactericides (2), as antiviral and antitumor agents (3), and as mucolytic agents in mouthwash (4). They are extensively used in industry as wet strength paper additives, flocculants, and modifiers for adhesives and coatings (5).

Polyethyleneimine is a water-soluble polyamine formed by the acid-catalyzed polymerization of ethyleneimine (6). Polyethyleneimine prepared in this manner contains primary, secondary, and tertiary amine nitrogens in a 1:2:1 ratio (5). The result is a highly branched spheroid-shaped molecule such as Structures I and II. A linear polymer (IV) of polyethyleneimine is also available *via* a two-step synthesis (7) and contains essentially only the secondary amine functionality if n is large. This linear polymer is crystalline and essentially insoluble in water at 25°.

In view of the therapeutic and hygienic usefulness of the commercial polyethyleneimines and the lack of literature



I: R = H, mol. wt. 40,000–60,000
II: R = H, mol. wt. 600
III: R = $COCH_3$, mol. wt. 40,000–60,000

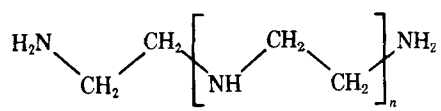


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Table I—Milligrams of Dye Retained (per Group of Three Rats[a]) *versus* Dose of I

Dose, mg/kg	Hours after Dosing 1[b]	2[c]	4[d]
250	7.50	5.50	3.98
	7.70	5.35	3.32
Average	7.60	5.43	3.65
125	4.67	3.05	1.08
	5.34	2.85	1.21
Average	5.01	2.95	1.15
62.5	5.55	1.28	0.65
	4.13	1.38	0.92
Average	4.84	1.33	0.79
31.2	2.35	0.73	0.10
	2.22	0.78	0.34
Average	2.29	0.76	0.22
15.7	1.07	0.68	0.38
	1.02	0.85	0.14
Average	1.05	0.77	0.26
0	1.17	0.40	0.08
	1.07	0.35	0.04
Average	1.12	0.38	0.06

[a] Six rats were used at each dosage level. Tabulated data refer to pooled values of these levels with two sets of three rats per level. [b] Zero-time control values: 5.75 and 7.25 (mean = 6.50). [c] Zero-time control values: 6.95 and 7.75 (mean = 7.35). [d] Zero-time control values: 5.70 and 6.70 (mean = 6.20).

Table II—Effect of Various Doses of I on Gastric Emptying Using the Phenolsulfonphthalein Method at Three Posttreatment Times[a]

Dose, mg/kg	Gastric Inhibition, % 1 hr	2 hr	4 hr
250	99.7 ± 1.5[b]	68.6 ± 1.0[b]	57.9 ± 5.3[c]
125	59.8 ± 5.2[c]	35.0 ± 1.4[b]	17.5 ± 1.0[b]
62.5	57.2 ± 1.1[d]	12.9 ± 0.7[b]	11.7 ± 2.2[d]
31.2	17.9 ± 1.0[c]	4.8 ± 0.3[c]	2.6 ± 1.9
15.7	−1.0 ± 0.4	5.2 ± 1.2[d]	3.2 ± 1.9
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] The samples consisted of two groups of three rats in each instance. Values are expressed as the mean ± *SE* of the mean. [b] $p < 0.005$ when compared to control groups. [c] $p < 0.025$ when compared to control groups. [d] $p < 0.05$ when compared to control groups.

reports concerning their effects on the gut, a study was conducted to determine the effects of ingestion of these polymers upon GI motor activity.

EXPERIMENTAL

Methods—Three hundred and twenty-four female Wistar rats, 144–155 g, were used. The first series of experiments was designed to determine the influence of polyethyleneimine mol. wt. 40,000–60,000[1] (I) upon gastric emptying; the phenolsulfonphthalein method (8) was used. Essentially, overnight fasted rats were intubated in groups of six with a predetermined dosage of I (adjusted to pH 7.0 with dilute hydrochloric acid) for each group along with 3 mg of phenolsulfonphthalein, a nontoxic, nonabsorbable dye.

Compound I and the dye were given in 1.5 ml of aqueous solution. At fixed time intervals, the animals were sacrificed and the residual phenolsulfonphthalein stomach content was determined by spectrophotometry. Control groups of animals received only dye and were sacrificed

[1] PEI 600, Dow Chemical Co., Midland, Mich.

Table III—Evaluation of the Rate of Onset of the Gastric Emptying Inhibitory Effect of I

Treatment	Time of Sacrifice after Phenol-sulfonphthalein Dosing, min	Gastric Dye Content in Milligrams at Time of Sacrifice; Dye Dosed at Indicated Times after Water (Control) or I (Treatment)[a]		
		0 min	15 min	30 min
Control (water)	0	5.8	7.75	7.05
	60	0.90	1.55	1.30
% Retention (60 min)	$= \frac{\text{mg (60)}}{\text{mg (0)}} = A$	15.5 ± 4.3	20.0 ± 1.0	18.4 ± 3.9
I (125 mg/kg)	0	7.65	8.40	8.40
	60	6.15	6.70	5.30
% Retention	$= \frac{\text{mg (60)}}{\text{mg (0)}} = B$	80.4 ± 7.2[b]	79.8 ± 1.2[c]	63.1 ± 3.6[b]
% Inhibition	= % retention with drug − % retention with water = B − A	64.9 ± 8.4	59.8 ± 1.5	44.7 ± 5.3

[a] Values are expressed as the mean for two groups of three animals. Retention and inhibition values are expressed as the mean ± *SE* of the mean. [b] $p < 0.025$ when compared to control group. [c] $p < 0.005$ when compared to control group.

similarly. Each group of six rats, including the control group, was divided into two sets of three rats each. The milligrams of dye was determined for each pooled set of three rats, and the group average was determined from the two sets of three rats in each group. Thus, group means were compared.

Zero-time controls also received only dye but were sacrificed immediately after dosing (t_0). This number is important since some dye is forced mechanically past the pyloric sphincter on dosing. Therefore, the actual dye at zero time must be determined. This phenomenon was confirmed by injecting laparotomized, anesthetized rats and observing the dye passage. Animals should be dosed gently. Stomach retention and percent inhibition of stomach emptying were calculated as follows:

$$\% \text{ retention } (t_1) = \frac{\text{mg of dye at } t_1 \text{ (treated)}}{\text{mg of dye at } t_0 \text{ (zero-time control)}} \times 100 \quad \text{(Eq. 1)}$$

$$\% \text{ inhibition } (t_1) = \% \text{ retention } (t_1) - \frac{\text{mg of dye at } t_1 \text{ (control)}}{\text{mg of dye at } t_0 \text{ (zero-time control)}} \times 100 \quad \text{(Eq. 2)}$$

The percent inhibition is the percent retention corrected for the percent of dye that remains at time t_1 in untreated, control animals. Table I provides the actual amount of dye determined at each dosage level, including zero-time and timed controls. These data show the reproducibility of the data and provide a basis for calculating the retention and inhibition values reported in Table II.

In the second series of experiments, designed to compare the phenolsulfonphthalein method with a bead method (9), 1.0 ml of the polyamine solution, again adjusted to pH 7.0, was administered to rats by intubation. This time, however, the polymer dose was immediately followed by a second solution (0.5 ml) containing a suspension of 30 very small resin beads of uniform diameter (0.5 mm) and shape. Each dosage group consisted of six animals. For the bead studies, the bead content was determined for each animal and was averaged for each group of six. The phenolsulfonphthalein comparison groups were analyzed as described.

The animals were sacrificed after fixed time periods (t_1), and the locations of the beads were noted. The number of beads leaving the stomach in a specified time was assumed to be a measure of stomach emptying. The percent retention and inhibition were calculated after referring to the appropriate control groups in a manner similar to that used in the phenolsulfonphthalein method:

$$\% \text{ retention } (t_1) = \frac{\text{number of beads at } t_1 \text{ (treated)}}{\text{number of beads at } t_0 \text{ (zero-time control)}} \times 100 \quad \text{(Eq. 3)}$$

$$\% \text{ inhibition } (t_1) = \% \text{ retention } (t_1) - \frac{\text{number of beads at } t_1 \text{ (control)}}{\text{number of beads at } t_0 \text{ (zero-time control)}} \times 100 \quad \text{(Eq. 4)}$$

Table IV—Comparison of Phenolsulfonphthalein and Bead Methods of Estimating the Effect of Various Doses of I on Gastric Emptying at 4 hr Posttreatment

Dose, mg/kg	Inhibition (Phenol-sulfonphthalein), %	Inhibition (Beads)[a], %
250	60.7 ± 5.0[b]	93.2 ± 4.8[c]
125	17.0 ± 1.1[c]	45.2 ± 12.2[b]
62.5	12.4 ± 1.9	29.3 ± 12.9
31.2	5.4 ± 1.9	15.8 ± 11.3
15.7	4.6 ± 1.6	22.0 ± 7.0[b]
0	0.0 ± 0.0	0.0 ± 0.0

[a] The samples consisted of six rats in each instance. Phenolsulfonphthalein samples were pooled as before, $n = 2$. Values are expressed as the mean ± *SE* of the mean. [b] $p < 0.025$ when compared to control groups. [c] $p < 0.005$ when compared to control groups.

In the next series of experiments, designed to determine the rate of onset of gastroinhibitory effects, 12 sets of three female rats were fasted for 24 hr. Zero-time (treated) animals were given 1.5 ml of a solution containing 125 mg of I/kg (pH 7) plus 3 mg of phenolsulfonphthalein. Fifteen- and thirty-minute animals were first given I at time zero in 0.75 ml of water and then, after the appropriate time, were given an additional 0.75 ml of a second solution containing 3 mg of the dye. Control groups of animals at 0, 15, and 30 min were treated in the same way except that they received pure water instead of the polymer solution.

All animals in this experiment were sacrificed 1 hr after dye administration, and the dye content of the stomachs was determined. The stomach contents of each group of three rats were pooled; that is, each group of three rats received a total of 9 mg of phenolsulfonphthalein. The results in Table III indicate the milligrams of dye remaining for each pooled set of three rats.

The next series of experiments was designed to evaluate the duration of the effect of I. Sets of six rats were each dosed with 125 mg/kg of the polymer and the dye marker. The same analytical techniques as already described were used; the groups were sacrificed at various time periods up to 6 hr after dosing.

Materials—Compound I was dialyzed for 30 hr in regenerated cellulose tubing[2] (molecular weight cutoff of 12,000) prior to use. A change of tubing was made after approximately 15 hr. Polyethyleneimine mol. wt. 600[3] (II) was used without dialysis. $\text{Acetyl}_{0.25}$ polyethyleneimine (III) was prepared from I and acetic anhydride (0.33 equivalent) in water at 35° according to literature procedures (6). It was dialyzed for 48 hr prior to use. Analysis by NMR spectroscopy in deuterium oxide indicated only primary amine acetylation: a broad singlet at 1.9 ppm ($NHCOCH_3$) and no signal at 2.1 ppm ($NRCOCH_3$). Linear polyethyleneimine mol. wt. 10,000[4] (IV) was used without dialysis.

RESULTS AND DISCUSSION

Dosages of 15–250 mg of I/kg to fasted rats gave dose-related, long lasting, but reversible, inhibition of stomach emptying. The effect of I on gastric emptying performance was defined by the time-dependent percentage loss of either inert beads or phenolsulfonphthalein from the stomachs of rats dosed orally with the polyamine (Tables II and IV).

These data indicate that the two techniques gave qualitatively com-

[2] A. H. Thomas Co., Philadelphia, Pa.
[3] PEI 6, Dow Chemical Co., Midland, Mich.
[4] Supplied by Dow Chemical Co.

Table V—Duration of the Delay of Gastric Emptying by I

Dosage	Retention[a] of Phenolsulfonphthalein in Rat Stomachs at Predetermined Posttreatment Times, %			
	1 hr	2 hr	4 hr	6 hr
Control	17.2 ± 0.7	4.1 ± 0.7	2.0 ± 1.1	0.1 ± 0.1
125 mg/kg	99.0 ± 1.5[b]	67.6 ± 1.9[b]	22.3 ± 3.4[c]	9.5 ± 3.0

[a] Calculations: (mg of dye remaining in stomach × 100)/(mg of dye initially administered). Samples consisted of two groups of three rats. Values are expressed as the mean ± *SE* of the mean. [b] $p < 0.005$ when compared with control groups. [c] $p < 0.05$ when compared with control groups.

parable results. The phenolsulfonphthalein method was quicker, easier, and more reproducible, and it gave lower values than the bead method. With the phenolsulfonphthalein method of estimating gastric emptying, an experiment was done to determine the onset of gastric inhibition, as indicated under *Experimental* (Table III).

In addition to the actual amounts of dye remaining at each of the three dosing times, Table III shows the percent retention and inhibition. Within the limits of the method, the percent inhibition at zero time after dosing was as great as at 15 min. At 30 min, the effect of the drug began to decrease. Thus, the data indicate that the onset of the effect of I must occur fairly rapidly following oral administration. The results in Table V indicate the duration of action of I at a dosage level of 125 mg/kg. Some effect was still observable 6 hr after dosing.

The LD_{50} for oral administration of I (pH 7) to the rat is approximately 2 g/kg[5]. At the dosages used for stomach retention, no signs of overt toxicity or pathology were observed over a pH range of 2–12.

In a recent article (10), it was reported that chemical trauma to the small intestinal mucosa prevents gastric emptying. Histopathological examination of the small intestine of rats treated with I showed normal tissue.

Preliminary retention experiments with a lower molecular weight branched polymer (II) suggest that the activity of this agent in producing a delay in gastric emptying may depend on molecular weight. A 250-mg/kg dose of II produced an inhibition of 82 ± 0.7% at 2 hr, in contrast with the value of 68.6 ± 1.0% observed for I.

Compound III did not inhibit gastric emptying in the rat as measured by the phenolsulfonphthalein method (inhibition of 2.0 ± 0.9%, 250 mg/kg, 4-hr evaluation). Likewise, IV had no observable effect when administered in the range of dosages that proved to be effective for I. These results suggest that large numbers of primary amine sites per molecule are required for optimum activity.

In summary, these results indicate that orally administered branched-chain polyethyleneimine is associated with delayed gastric emptying in fasted rats. With the method used, a dosage of 125 mg of I/kg produced this effect as early as 15 min following administration, and the effect could be detected up to 6 hr later.

[5] Unpublished data.

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